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STUDIES ON THE CONTROL OF THE ACIDITY OF THE GASTRIC JUICE[1]

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During the course of digestion, marked variations in the concentration of the free hydrochloric acid of the gastic juice occur. Such variations raise the still open question as to the mechanism of control of the acidity of the juice. Are the fluctuations in the concentration of free acid determined by a regurgitation of duodenal alkaline juices into the stomach as postulated by Boldyreff (1907–1908) or are they determined by some other mechanism?

Boldyreff proposed the theory of the "self-regulation of the acidity of the gastric juice" by regurgitation of duodenal alkalis into the stomach, on the basis of a series of observations which extended over a period of several years. Among other facts he showed that when 200 cc. of 0.5 per cent hydrochloric acid were introduced into the stomach of a dog, the acid was neutralized by regurgitation into the stomach of duodenal alkalis, chiefly pancreatic juice. On the basis of his experiments he concluded that fresh gastric juice is secreted into the stomach with a high concentration of free hydrochloric acid, and that the acid stimulates regurgitation of duodenal alkalis into the stomach which regulates the acidity of the juice.

Clinical observers have sought to substantiate this theory by showing that the concentration of trypsin rises during the latter part of a fractional test-meal when there is a fall in the concentration of the acid. They interpret this increase in the concentration of trypsin as due to an increased regurgitation of the duodenal alkalis which act to neutralize the free acid.

[1] Abridgment of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfilment of the degree of Ph.D. in Surgery.

Bolton and Goodhart (1922) studied the problem from a clinico-pathologic viewpoint. They determined the concentration of acid and the inorganic chlorides after a test-meal in a series of normal persons, and in a series of persons with pyloric obstruction. They showed that whereas in the normal person the final fall in the free acid value was accompanied by a rise in the concentration of the chlorides, in the persons with pyloric obstruction there was a final rise in the free-acid concentration associated with a low chlorine value. They interpreted these inverted curves of acid and chlorides in the persons with obstruction as due to defective regurgitation of alkalis through the pylorus and consequently to defective neutralization of the acid.

Baird, Campbell and Hern (1924) presented evidence of a contrary nature from their studies carried out on normal persons. They determined the acid and neutral chlorine curves of the fractional aspirations of a test-meal in the normal person. They then eliminated the alkaline secretions of the duodenum by continual suction through a duodenal tube. While the alkalis were being aspirated in this manner they repeated the test-meal. Chemical studies of the aspirated specimen showed that there was an identical drop in the concentration of the acid and the same final rise in the neutral chlorides in both experiments. They interpreted these results as showing that the regurgitation of duodenal alkalis does not constitute the essential factor in controlling the acidity of the gastric juice.

My experimental studies were made to determine whether the total elimination of the duodenal secretions from beyond the pylorus by surgical methods, or the loss of pyloric alkaline mucus following resection of the antrum of the stomach, would alter the character of the response of the stomach to injection of histamine or to a test-meal of meat and water so far as the control of the acidity of the juice is concerned.

METHOD OF STUDY. Fractional gastric analysis was carried out by a method which I devised for use on the normal dog. Histamine and a test-meal of meat and water were the two stimuli of secretion used in the method of fractional analysis. Aspirations of the meal of meat were easily performed when the meat meal was in the stomach, but when only small quantities of juice were in the stomach, as after stimulation by histamine, the organ had to be inflated slightly through a pressure line connected with the aspirating mechanism before the specimens could be aspirated. In all the specimens the total acid value was titrated against tenth-normal sodium hydroxide, using 1 per cent alcoholic solution of phenolsulphonephthalein as the indicator; free acid was determined using Topfer's reagent as indicator; the total chlorides were determined by the Folin method for urine. In charting the values the chlorine equivalent of the free hydrochloric acid was deducted from the total chlorides so that the curve represents the combined or neutral chlorides.

Two series of studies were carried out. In the first series of observations the duodenal contents were eliminated by surgical procedures and the results of fractional analysis after operation compared with similar fractional analysis made before operation. In this way the influence of the duodenal juices on the phenomenon of acid control was measured. The operative procedure consisted in a slight modification of Mann's method of surgical duodenal drainage. The bowel was divided just below the pylorus and also just below the ligament of Treitz. This gave an isolated loop of duodenum receiving the bile and pancreatic juices. The proximal end of the loop was closed; the distal end was anastomosed to the distal

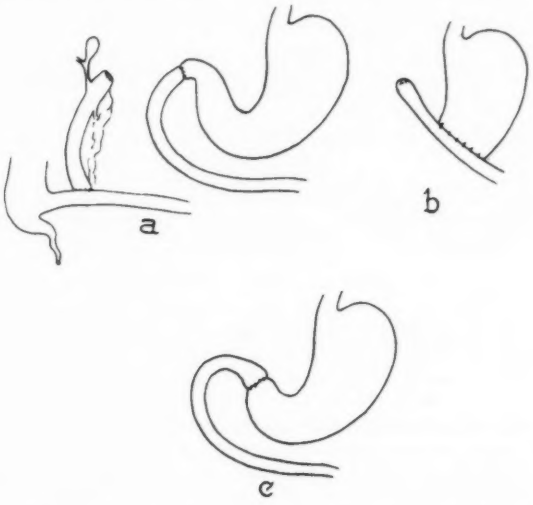

Fig. 1. Type of operative procedures used to localize the mechanism for controlling the acidity of the gastric juice.

portion of the ileum, thus draining all the significant alkalis away from the pylorus into the distal portion of the ileum. The open end of the jejunum was then anastomosed to the small segment of duodenum which was left at the pylorus after the lumen of each segment had been enlarged by a longitudinal incision; thus the stomach with its nerves and blood supply intact, and without any mechanical impediment to emptying, expelled its contents directly into the jejunum. The possibility of regurgitation of any of the duodenal alkalis back through the 6 meters of bowel, against the current of an oncoming meal, is obviously impossible. Bile was never observed in the stomach after this operative procedure (fig. 1, *a*).

In the second series of observations the operative procedures consisted in

complete and partial resection of the pyloric antrum. In complete resection of the pyloric antrum the stomach was transected at the pylorus and at a line 1 cm. orad to the incisura angularis. Antecolic anastomosis of the fundus and jejunum was carried out (fig. 1, *b*). This was done to measure the influence of the loss of the alkaline mucus of the antrum on the control of the acidity of the gastric juice. As a control on the results of this procedure, partial resection of the pyloric antrum was carried out. The stomach was transected at the pylorus, and at a line about 2.5 cm. orad to the pylorus. This procedure duplicated the operative derangement of total resection of the antrum; the same nerves and blood vessels were severed with the same degree of trauma. However, a wide strip of intact pyloric antrum with its normal mucosa was preserved (fig. 1, *c*).

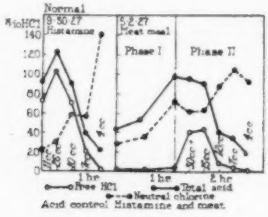

Fig. 2

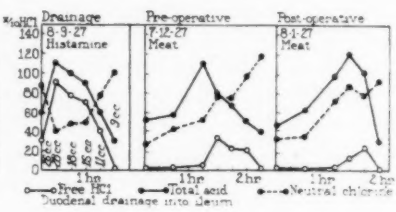

Fig. 3

Fig. 2. *Histamine:* the relationship between the rate of secretion and the degree of acidity is shown; final high neutral chlorides with neutralization. *Meat:* two phases of digestion; same mechanism for control of acid in the second phase as with histamine.

Fig. 3. Alkalis eliminated from beyond the pylorus. *Histamine:* same character of curve with same features active in controlling acidity as in the normal dog. *Meat:* same character of curves before and after drainage; the stomach's independence of the duodenal alkalis in controlling the acid is shown.

RESULTS IN A NORMAL DOG. *Histamine* (fig. 2). One milligram of histamine injected intramuscularly caused the appearance of free hydrochloric acid in the stomach within fifteen to thirty minutes after injection. Aspirations were made at fifteen-minute intervals after the injection. The walls of the stomach were collapsed and the quantities of juice were so small that the stomach had to be slightly inflated before each aspiration. The successive aspirations showed a rapid increase in the concentration of free acid. The highest concentration (80 to 105 cc. in terms of tenth-normal acid for each 100 cc.) was usually attained on the second to fourth aspiration. Subsequent aspirations showed rapid reduction in the concentration of the acid until the point of neutralization was reached.

With this changing acid curve, three variable factors were observed which always maintained a constant relationship to the changing acid

concentration, and to each other. The first factor was the rate of secretion of the juice. When the aspirations were made every fifteen minutes, the total quantity of juice in the stomach was withdrawn. Taking the quantity of juice aspirated every fifteen minutes as a rough index to the rate of secretion, it was found that a rise in the concentration of acid was associated constantly with an increase in the rate of secretion, and that a fall in the concentration of acid was associated with reduction in the rate of secretion of the juice.

The second variable factor was the physical character of the secreted juice. In the resting stomach in which there was no free hydrochloric acid, a small amount of mucus was continually secreted. After stimulation by histamine as the concentration and rate of secretion of the juice increased the aspirated specimens changed successively from this mucoid type of secretion to a thin, watery clear secretion. With a decrease in the concentration and rate of secretion of the acid, the successive aspirations showed a reverse change from the thin, watery, clear secretion to an opaque mucoid type.

The third variable factor was the concentration of the neutral chlorides. This constantly showed an inverse relationship to the changes in the concentration and rate of secretion of the juice, and to the associated changes in the physical character of the juice. As the concentration and rate of secretion rose, and the mucoid juice changed to a clear, watery secretion, there was an inverse fall in the concentration of the neutral chlorides; as the concentration and rate of secretion of the acid fell, and the mucoid type of secretion reappeared, there was an inverse rise in the neutral chlorides (best seen in fig. 3). These three variable factors always exhibited this constant interrelationship. The total acid value was always 20 to 40 more than the free acid value. When the two values were charted, the curves were always parallel. The mucus of the resting stomach was never alkaline, but always had a combined acid value of 20 to 40.

Meal of meat (fig. 2). The meal of meat consists of 80 grams of lean, finely ground horse meat mixed with 250 cc. of distilled water. Fractional aspirations revealed two distinct phases in the digestion of the meal. In the first phase, during which 30 cc. quantities of the meal were aspirated every thirty minutes, there was a progressive disintegration of the meal to a homogeneous fluid state. With this alteration there was a parallel rise in the concentration of the total (combined) acids, and the neutral (base) chlorides. Hydrochloric acid was absent during this phase or appeared only at the end of the phase. Apparently the acid, which was secreted at a concentration of 0.6 per cent free acid, as shown by Pavlov's studies with gastric pouches, combined with the meat to give an acid protein and a base chloride, so that this phase may be called the acid (food) combining phase. When the total acids reached their highest concentration and the meal was

wholly disintegrated, the stomach had usually emptied itself completely of chyme, and hydrochloric acid appeared in free form.

The appearance of hydrochloric acid in free form usually coincided with the beginning of the second phase. In this phase the quantities of juice secreted were small and the organ was collapsed, so that during this phase the stomach had to be inflated slightly before each aspiration. During this phase the aspirations were made at fifteen-minute intervals, and each time the stomach was completely emptied. There was a rapid rise in the concentration of the free acid to its maximal value. From this high point the values of the free acid fell rapidly with the successive aspirations to the neutral point. Again there was the same interrelationship of the three variable factors to the fall in the concentration of acid. It was associated with a fall in the rate of secretion (as indicated by the decreasing quantities of juice aspirated every fifteen minutes), and with transition from a thin, watery clear secretion to a mucoid type of secretion. There was the usual inverse rise in the concentration of the neutral chlorides to a maximal value. Because in this phase there is a reduction in the concentration of free acid to the point of neutralization, it is called the acid-control phase.

Bile was observed in about 4 per cent of 1500 single specimens aspirated in the course of 285 complete fractional studies on normal dogs. It was observed more frequently in the studies with histamine than with meat. After the test-meal of meat bile was observed only during the second or acid-control phase, when the chyme had been emptied from the stomach, and the prepyloric segment was relaxed. The work of Wright and Medes suggests that pancreatic juice may regurgitate into the stomach even more frequently than bile. These facts raise the question as to the significance of these regurgitated alkalis in controlling the acidity of the gastric juice.

Results in the first series of observations. From 4 to 120 days after surgical duodenal drainage the studies which had been made before operation with histamine and the meat meal were repeated. In response to stimulation by histamine these dogs with the duodenal alkalis eliminated repeated the same phenomena that they showed before operation. Aspirations made every fifteen minutes showed a rapid rise in the concentration of acid to the same average levels observed before the duodenal alkalis were eliminated. This again was associated with identical changes in the three variable factors: an increase in the rate of secretion, a change from a mucoid to a watery, clear secretion, and an inverse fall in the curve of the neutral chlorides. With falling concentration of the juice to the point of neutralization there occurred, as in the normal dog, an associated reduction in rate of secretion, a change back to the mucoid type of secretion, and an inverse rise in the concentration of the neutral chlorides to a maximal value (fig. 3).

The response to the test-meal of meat was identical after operation with

that noted before operation. There was the acid-combining phase characterized by progressive increase in the fluidity of the chyme, and a parallel rise in the value of the total acids and neutral chlorides. The stomach emptied itself progressively and when completely emptied the second or acid-control phase supervened. There was a rapid rise in the concentration of the acid to normal heights. This was followed by a fall in the concentration of acid to the point of neutralization. This was associated, as before operation, with a reduction in the rate of secretion, a transition from a watery to a mucoid type of secretion, and an inverse rise in the neutral chlorides to a maximal value (fig. 3).

Fasting stomach. Hourly aspirations were done for a period of twenty-four hours on four fasting dogs in which the duodenal alkalis had been eliminated by surgical duodenal drainage. The same adequate control of the acid was shown as in a similar study of normal dogs. Small quantities of mucus (4 to 6 cc.) were recovered every hour. There was usually an absence of free hydrochloric acid, a low combined acid value, and a maximal neutral chlorine value. When free acid did appear occasionally, it was quickly neutralized as in the normal dog (fig. 4).

Carbohydrates. It is possible that food substances other than protein cause a significant regurgitation of alkalis which might influence the acidity of the gastric juice. This possibility was studied by adding carbohydrate to the usual meal of meat and water, and comparing the resultant curves on the normal dog with those of a dog in which the alkalis had been eliminated by the drainage operation. First, the usual fractional study with the meat-water meal was done on both dogs. Then the meal was altered by replacing half the water content by an equal quantity of karo syrup. This gave a meal with the usual meat and fluid volume, and with an additional carbohydrate factor. The fractional studies after this modified meal showed the same changes in the curves in both the normal and operated dog. In both instances there was an identical marked prolongation of the first or acid-combining phase to almost twice its usual duration. This change was characterized in normal dogs and in dogs operated on by a depression of the total acid curve to a flat level below that of the neutral chlorine curve. The curve for free acid did not change significantly; it fell within the range of normal variations. The final neutralization of the free acid was associated in both instances with a fall in the rate of secretion, and a transition to a mucoid type of juice. There was the usual final rise in the neutral chlorides.

Fats. The influence of fat on the regurgitation of duodenal juices was investigated in a similar manner. Half the fluid content of the usual meal of meat and water was replaced by an equal volume of olive oil. Comparison of the response to this test-meal, containing fat, with the response after the usual meat-water meal showed the same variations in the response in

the normal dog, and in the dog with the duodenal alkalis eliminated. The acid-combining phase was prolonged to twice its usual duration in both dogs, with a striking break at some point on the total acid curve. The free acid was not controlled any better in the normal dog than in the dog with the duodenal juices eliminated, even though fat may augment the factor of regurgitation in the normal dog. The acid was neutralized in both instances by the usual reduction in the rate of secretion and transition to a mucoid type of secretion with the terminal rise of the neutral chlorides.

The results of these studies showed that under vastly different conditions of activity, the phenomena of digestion and of the control of the acidity of the gastric juice are constant, whether or not the duodenal alkalis can regurgitate into the stomach. They indicate that when regurgitation does occur it is apparently an associated phenomenon depending, first on the periodic

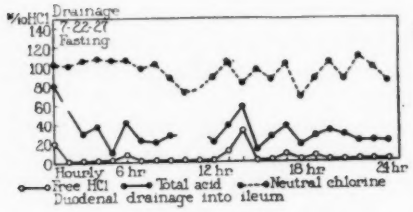

Fig. 4

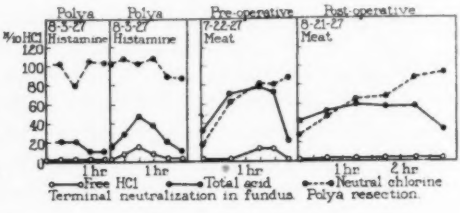

Fig. 5

Fig. 4. Alkalis eliminated. Complete control of the acidity is shown for a fasting period of twenty-four hours, with absence of alkalis beyond the pylorus.

Fig. 5. *Histamine:* the two curves show loss of significant acidity after resection of prepyloric segment; free acid usually absent but occasionally present in low concentration. *Meat:* alteration of the curve from normal following resection of the prepyloric segment; slow partial digestion of meat, limited rise in total acids, and loss of free acid.

activity of the digestive glands, and second, on the relaxation of the pyloric antrum toward the end of digestion and during rest, which would permit regurgitation of the alkaline juices. These results suggest that the control of the acidity of the gastric juice depends essentially on some intragastric mechanism.

RESULTS IN THE SECOND SERIES OF OBSERVATIONS. The preceding studies localized the control of the acidity of the gastric juice to a mechanism within the stomach. Some investigators have interpreted the changes in the chlorides as a shift from an acid-chlorine secretion to a base-chlorine secretion in the acid-producing cells of the stomach. If the curve of the neutral chlorides be taken as an index to the neutralization of the acid, its close interrelationship with the changes in the rate of secretion and the alterations from a watery to a mucoid type of secretion, suggests that these two factors are involved in the mechanism.

If mucus plays a significant part in controlling the acidity of the juice, is this mucus derived from the fundus or from the antrum of the stomach? Heidenhain first showed that the secretion from the pyloric antrum is slightly alkaline, whereas the mucus aspirated from the stomach always has a combined acid value of 20 to 40. This has been amply verified by later investigators.

The antrum was completely resected in five dogs to measure the influence on the acidity of the juice of the loss of the alkaline mucus secreted by this segment (fig. 1, *b*). But the procedure threw important light on the other factor associated with the control of the acid, that is, the changes in the rate of secretion of the acid.

The usual preoperative and postoperative studies with histamine and the test-meal of meat and water were made on these five dogs. After resection of the antrum the usual typical responses were no longer observed. Injection of histamine did not cause the usual outpouring of highly concentrated juice, as occurred in the normal dog. Free hydrochloric acid usually was not found; in a few instances a weakly acid juice of a value of 16 was aspirated. Instead of the usual increasing quantities of juice which were aspirated in the normal dog evey fifteen minutes, only small amounts of mucoid secretion (5 to 6 cc.) were recovered after the antrum had been resected. The aspirated specimens were identical with those recovered from the stomach of a normal dog during the resting period when free acid was absent, that is, there were small amounts of mucus with a low total acid value and a maximal neutral chlorine value (fig. 5).

In response to the meal of meat and water, there was very slow, incomplete disintegration of the meat after the antrum had been resected. There was a prolonged acid-combining phase with a very gradual and limited rise in the total acids. When the stomach was completely emptied there was no typical second phase, with the appearance of free hydrochloric acid. Instead the aspirated specimens consisted of small quantities of mucus (5 to 6 cc.) with a low total acid value and a maximal neutral chlorine value, which are the distinctive phenomena of the normal resting stomach after the free acid has been neutralized (fig. 5).

This type of response to a meal of meat can only be explained on the basis of failure of the usual increase in the rate of secretion of acid, as was found after the use of histamine. These changes in the character of the response to histamine and meat after total resection of the antrum indicate that there is an essential relationship between the changes in the rate of secretion of the juice and the integrity of the prepyloric segment.

As a control study on this observation, a limited resection of about 2.5 cm. of the antrum adjacent to the pylorus was done on four dogs. This left a wide strip of the antrum with its normal mucosa intact (fig. 1, *c*). After this operative procedure the usual type of response to stimulation was

observed. A test-meal of meat showed all the characteristic phenomena observed in the normal dog, dependent on an increase in the rate of secretion of acid. There was an acid-combining phase with a rise in the total acid and neutral chlorides; also an acid-control phase with the appearance and subsequent neutralization of the free acid (fig. 6). This fact suggests that the loss of the secretory response in dogs after total resection of the antrum is dependent on the loss of the prepyloric segment; conversely, the rise in the rate of secretion in the normal dog is probably dependent on the integrity of some portion of the prepyloric segment.

If the curve of the neutral chlorides can be taken as an index to the neutralization of acid of the stomach, these last two studies indicate that it is probably accomplished by the mucus secreted in the fundus. The dogs in which the antrum had been partially resected did not show alterations in the efficiency of the mechanism for controlling the acidity of the gastric juice, after the loss of a good portion of the alkaline mucus secreted by that segment so the antral mucus probably is not the neutralizing factor. Furthermore, after resection of the whole antrum, the secretion from the fundus had every feature characteristic of the secretion from the normal resting stomach when the acid was neutralized. There were small amounts of mucus that had a low combined total acid value of 20 to 40 and a maximal neutral chlorine value. The characteristic curves noted on normal dogs after histamine stimulation have been observed by Close, and by MacLean and his co-workers, in isolated gastric pouches made in the fundus of the stomach. This duplication of the neutralization of the acid in a fundic pouch with the secretion of the distinctive type of mucus by the fundus, as shown in these studies, indicates that this segment is probably the source of this significant secretion of mucus.

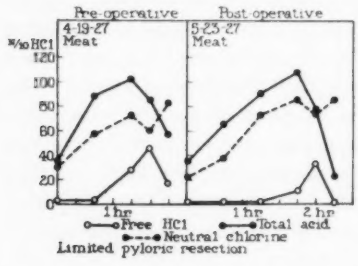

Fig. 6. Partial resection of pylorus below incisura angularis; persistence of characteristic secretory response to meat when part of the prepyloric segment is preserved distal to the incisura is shown.

COMMENT. *Method.* The method of fractional gastric analysis which I devised permitted me to study the physiology of the intact stomach of normal dogs. The friendliness of the dogs and the uniformity of the results indicate that there was no important psychic reaction to the procedure. The use of histamine permitted me to study the activity of the stomach while it was secreting pure gastric juice. The meal of meat and water gave a measure of the course of events during normal digestion. Meat

is the food with which intragastric digestion is chiefly concerned, stimulating all the secretory and motor activities of the stomach.

Regurgitation. The first series of experiments in which the duodenal alkalis were eliminated showed that regurgitation of duodenal alkalis into the stomach does not constitute the mechanism for regulating the acidity of the gastric juice. Yesko (1928) confirmed these results in later experiments with reference to the pancreatic juice. It appeared from my studies that the mechanism which controls the acidity of the gastric juice is essentially an intragastric one. The constant relationship of the inverse curves of free acid and neutral chlorides to the observed changes in the rate of secretion and the transition from a watery to a mucoid type of secretion, indicates that these two factors are probably of significance in regulating the acidity of the juice.

Rate of secretion. The relationship between the concentration of the free hydrochloric acid and the rate of secretion was indicated by a rough method of measurement in these studies. However, the results agree with Pavlov's observations with gastric pouches: "It is a rule almost without exception, that the acidity of the juice is closely dependent on the rate of secretion; the more rapid the latter the more acid the juice and vice versa." In these studies the dogs with the antrum resected did not manifest an increase in the rate of secretion of the juice or an increase in the concentration of the acid. These results indicated the importance of the prepyloric segment in determining the acidity of the juice in the normal stomach, not because of its alkaline secretion, but because it is a significant link in regulating the rate of secretion of acid.

Mucus. The constant association of mucus secreted by the stomach with these changes in the acid and chlorides has been emphasized because of its possible action in bringing the free acid to the point of neutralization. In the resting stomach the volume of acid secreted is so little that it apparently combines with the basal secretion of mucus to give an organic acid and a base chloride. This cannot be accounted for on the basis of a reaction between an acid and an alkali, because of the weakly alkaline character of mucus. It is probably dependent on a protein-acid combining phenomenon, as suggested by Foster's (1907) experiments. He showed that mucus scraped from a pig's stomach had a marked combining affinity for free hydrochloric acid when incubated at body temperature, in the presence of pepsin. In the absence of pepsin this affinity was minimal. Since he had used mucus which had already combined with some acid in the stomach, he concluded: "It is probable also that in the stomach the combining power of fresh mucus as it is secreted from the glands is much greater than these experiments indicate."

The mode of this combination is probably analogous to that observed with the meal of meat. The acid which is poured out in response to the

stimulus of meat combines with the protein to give an increasingly concentrated acid protein and neutral (base) chloride. The mucus secreted by the resting stomach is a protein, and in the presence of pepsin probably combines with the small amount of acid secreted by the resting stomach to give an acid mucin, and a neutral (base) chloride. This hypothesis also accounts for the fact that the secretion of mucus in the fundus is always acid, whereas mucus elaborated elsewhere in the body is alkaline. This significant fact should be accounted for by any other hypothesis of the method for controlling the acid of the gastric juice.

Mechanism of acid control. The mechanism of acid control suggested by these studies may be described as follows: In the resting state the fundus of the stomach probably secretes a constant basal amount of mucus as indicated by the studies of Ivy (1919) and of Ma, Lim and Liu (1927). A small amount of hydrochloric acid of a concentration of 0.5 to 0.6 per cent is also secreted which combines with the basal secretion of mucus in the presence of pepsin, giving an acid mucin and a neutral (base) chloride of maximal concentration. Through the mediation of some portion of the prepyloric segment, the rate of secretion rises gradually, changing the juice from a mucoid to a clear watery type of secretion. As the rate of secretion rises, there is an increasing volume of acid that is uncombined by the mucus, which tends to approximate a maximal concentration indicated by the value of the total chlorides. The constant fraction of secreted acid combined with the mucus as a neutral chloride is diluted by the increased volume of juice, and its concentration falls.

As the rate of secretion of acid falls there is proportionately less of the acid uncombined with the mucus, and its concentration falls proportionately. The constant fraction of the volume of acid combined with the basal secretion of mucus as a neutral (base) chloride is progressively less diluted and consequently increases in concentration as the character of the secretion changes back to the mucoid type. The rate falls to the point where the low residual secretion of acid is again combined with the mucus as acid mucin and base chloride.

After the test-meal of meat the stimulus through the antrum to the secretion of acid is gradually reduced as the meat is digested and disintegrated. When the digested meat has passed from the stomach there persists only a moderately elevated rate of secretion. The free acid then appears in concentration proportionate to this residual elevation of the rate of secretion. The rate falls until it reaches the low volume of the resting stomach at which rate it combines completely with the basal secretion of mucus (in the presence of pepsin) giving a combined acid and a neutral (base) chloride.

Extraneous factors. The mechanism described probably determines the acidity of the juice directly as it leaves the fundic glands. There are other

factors which probably influence the concentration of acid in a specimen of juice aspirated from the stomach. If a considerable quantity of highly concentrated juice rests in the stomach, the rate at which it is emptied will determine the extent to which a less concentrated juice from the fundus, due to a falling rate of secretion, will determine the acidity of the whole gastric contents.

If the acid juice rests in the stomach for any considerable time, it might be neutralized to some extent by the alkaline mucus secreted by the antrum, and by any duodenal alkalis which might regurgitate into the stomach. However, these factors do not constitute an essential part of the mechanism for controlling the acidity of the gastric juice. They are variable and associated phenomena which do not have any demonstrable influence on the changing concentration of the gastric juice as it is secreted from the fundic glands into the lumen of the stomach.

SUMMARY

I devised a method for doing fractional gastric analysis on the normal dog. Histamine and a test-meal of meat and water were used to stimulate the secretion of acid. The uniformity of the results give value to the method in studying the physiology of the stomach.

In the first series, seven dogs were subjected to the surgical procedure of duodenal drainage. By this operation all the duodenal alkalis were shunted away from the pylorus into the distal portion of the ileum. Post-operative fractional analysis did not show variations from the curves found before operation so far as they represented the chemistry of digestion, or the control of the acidity of the juice. The addition of carbohydrate and fat to the protein meal showed the same alterations of the curves from a meal of meat and water in normal dogs and in dogs operated on. The final control of the acid was not altered. Regurgitation of the duodenal alkalis is not, therefore, the essential mechanism for controlling the acidity of the gastric juice, but is probably an associated phenomenon.

The results showed that after the acid has stopped combining with the food, the mechanism for controlling the acidity of the juice consists probably of two factors capable of bringing the juice to complete neutralization. The first factor is a gradual reduction of an established high rate of secretion of the acid to a low rate of secretion. The changes in the rate of secretion as shown in the second series are dependent in some fashion on the integrity of some portion of the prepyloric segment.

A second factor suggested by the results appears to be the capacity of the constant basal secretion of mucus to combine with a constant fraction of the changing volume of acid secreted. This mucus is probably derived from the adjacent mucus-secreting glands in the fundus of the stomach. When the rate of secretion of acid has fallen to the low basal rate of the rest-

ing stomach, the amount secreted apparently is so small that it completely combines with the basal mucous secretion giving a combined acid and a neutral (base) chloride.

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STUDIES ON THE EMPTYING OF THE STOMACH[1]

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A half-century of experimentation has not solved the problem of the emptying of the stomach. Many factors which might influence the process, as acid, alkali, fluidity, osmotic pressure, and temperature, have been investigated, but in any method of investigation the question always presents itself of how nearly the conditions of the experiment approach those of normal physiologic activity.

My purpose in undertaking this study was to apply to the investigation of the problem a method of fractional gastric analysis which I had devised for the normal dog. The correlation of roentgen studies with the method of fractional analysis enabled me to observe simultaneously the motor and chemical changes which were developing in the organ. The animals were normal healthy kennel dogs, and extraneous factors, such as anesthesia or operation, which might influence the results were not introduced. The quick passage and withdrawal of the stomach tube used in the fractional studies were accomplished with very little apparent psychic effect.

Two series of studies were carried out. In the first series roentgen-ray examinations of the stomach were made to determine the rate of emptying and the type of motor activity at distinct points in the course of digestion. In the second series, the influence of certain factors associated with the process of emptying, namely, free hydrochloric acid, fluidity, and products of digestion was investigated to determine whether any one of them might be the essential factor which controlled the emptying of the stomach.

METHOD OF STUDY. A brief review of the method of fractional gastric analysis is necessary for understanding the sequence of the studies. A meal of meat and water (80 grams of ground horse meat and 250 cc. of water) was fed to a dog which had fasted for eighteen hours. Aspirations were made at regular intervals beginning half an hour after the meal had been eaten. Chemical studies of the aspirated specimens revealed two distinct phases to the digestive cycle. In the two or three specimens aspirated at

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half-hour intervals first there was a gradual rise in the values of the total acids and neutral chlorides. They rose from an early low level to a high level late in the phase. There was absence of free hydrochloric acid or else it began to appear at the end of the phase. These rising curves were associated with gradual disintegration of the meat to a completely fluid state. As the free acid secreted during this phase was combined with the protein, this was called the "acid-combining phase." During this period the aspirations were made at intervals of half an hour, and there were usually from two to four of them.

Shortly after the total acid curve of the first phase reached a maximal point, the stomach usually had emptied itself completely of its fluid

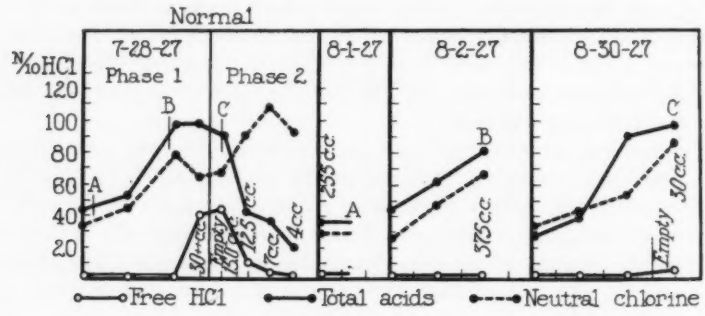

Fig. 1. Normal curve in response to a test-meal of meat. Roentgenograms were made at distinctive points, *A*, *B* and *C*. Chemical analyses of the specimens aspirated at the points at which roentgenograms were made, August 1, 2 and 30, are shown.

chyme and the second phase followed. During this phase only small quantities of clear juice, which contained free hydrochloric acid, could be aspirated. The specimens were aspirated at fifteen-minute intervals during this second phase. Successive aspirations showed decreasing volume. The free acid value rose quickly to a maximum and with the final aspirations fell to the point of neutralization. This second phase is called the "acid-control phase," because the free acid is brought to a point of neutralization by a mechanism discussed in a previous paper (fig. 1).

As these changes occurred in the cycle of digestion, alternations in the motor state of the stomach were suggested by three distinctive conditions encountered by the aspirating tube, within the stomach. Early in the first phase (the first distinctive point), when the digestion of the meat had hardly begun, the tube could not be inserted as far as in the later periods. It was frequently caught in a contracting segment of the stomach, suggesting that the distal end of the stomach was in a state of

vigorous contraction. Later in the first phase (the second distinctive point), when the stomach was distended with fluid chyme, the tube could be advanced farther, and there was not the same evidence of vigorous motor activity in the pyloric antrum. In the second or acid control phase (the third distinctive point), when the stomach contained only small quantities of free acid juice, the organ was collapsed and its walls approximated so that it had to be inflated slightly in order that the small quantities of juice might be aspirated.

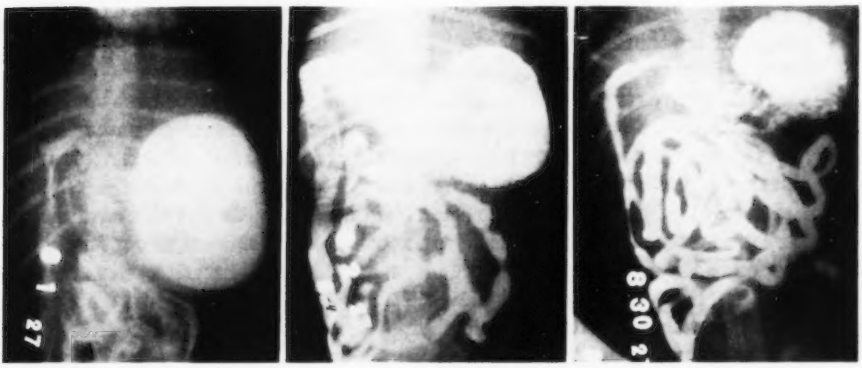

Fig. 2 Fig. 3 Fig. 4

Fig. 2. Roentgenogram taken at point *A*, of figure 1, early in the first phase of digestion. Small amount of emptying only a short while after feeding of the meal. Quantity of barium which emptied in a fifteen-minute period is shown.

Fig. 3. Roentgenogram taken at point *B*, of figure 1, late in the first phase of digestion. Large amount of emptying of disintegrated meal in fluid state with high total acids and relaxing pyloric antrum. Quantity of barium which emptied in a fifteen-minute period is shown.

Fig. 4. Roentgenogram taken at point *C*, of figure 1, at beginning of second phase. Almost complete emptying at point where chyme had left stomach; free acid was present, stomach was collapsed and pyloric antrum was relaxed. Quantity of barium which emptied in a fifteen-minute period is shown.

To measure the changing motor activity of the stomach, barium was introduced into the stomach at the three distinctive points in the cycle just described, and a roentgenogram was taken after fifteen minutes had passed. A comparison between the amounts of chyme that emptied into the intestine during the fifteen-minute interval at these three distinctive points in the cycle, gives a measure of the changing rate of empyting as digestion proceeds. Fluoroscopic observations were made to determine the changes in the motor state of the stomach at these three points in the cycle.

RESULTS IN THE FIRST SERIES OF OBSERVATIONS. *Early in first phase of*

digestive cycle. (*Fig. 1 A.*) The usual meal of meat and water was administered to a dog which had fasted for eighteen hours. Within fifteen minutes and before any marked digestion had begun, 50 cc. of the meal were aspirated. This was immediately replaced by 50 cc. of a mixture of barium with gum acacia, which kept the volume of the gastric contents unchanged. A roentgenogram was taken after fifteen minutes. Even at this early period some dogs emptied a small quantity of material past the pylorus (fig. 2). Fluoroscopic examination showed that the fundus was distended like a globe, containing most of the meal. It did not exhibit gross peristalsis, only a simple wavering around its circumference. The antrum, from the incisura to the pyloric sphincter, was in a state of constant tonic contraction appearing like a narrow tube with frequent vigorous peristaltic waves traversing it. Frequently two or three waves were visible at once between the incisura and the sphincter. This limited emptying and vigorous motor activity was associated with low total acid and neutral chlorine values, and an absence of free hydrochloric acid (fig. 1, *A*, 8–1–27).

Later in first phase of digestive cycle. (*Fig. 1, B.*) Similar observations were made late in the first phase. On another day, the usual meal of meat was fed to the dog, and three successive aspirations were made at intervals of half an hour. This carried the cycle to a point where the stomach was distended with brown opaque fluid chyme from the disintegrated meat. At the second point late in the first phase, 50 cc. of chyme were aspirated. This was immediately replaced by 50 cc. of barium mixture, again leaving the fluid volume unchanged. A roentgenogram taken after fifteen minutes demonstrated that considerable chyme was passing the pylorus every fifteen minutes (fig. 3). Fluoroscopic study showed that the whole prepyloric segment was considerably relaxed, and that there was less of the vigorous tonic and peristaltic activity which characterizes the early point in digestion. The duodenal cap filled periodically and emptied into the duodenum with very little motor activity in the prepyloric segment. The fundus was diminished in size and still without evidence of significant peristalsis. Chemical analysis of the aspirated specimens showed that there was a marked increase in the total acid and neutral chlorine values, and that the rapid emptying was taking place in the absence of free hydrochloric acid (fig. 1, *B*, 8–2–27).

Second phase of digestive cycle. (*Fig. 1, C.*) At a later date the usual meal of meat and water was administered to the dog. The usual aspirations were made until the point was reached where the stomach was first found to be empty of chyme, and its walls collapsed. The organ was inflated slightly and about 30 cc. of juice (the total content) were aspirated. This aspiration also removed all the air and the walls again collapsed. Chemical analysis of the specimen showed a total acid value of 98 cc. of tenth-normal acid and a free acid value of 8 cc. of tenth-normal acid (fig. 1,

C, 8–30–27). One hundred cubic centimeters of barium mixture with a free acid value of 8 was introduced into the stomach. A roentgenogram taken after a fifteen-minute interval showed that in this second phase most of the barium had passed into the intestine in this interval, and the stomach was nearly empty (fig. 4). Apparently at this point there was almost complete relaxation of the antrum which permitted the rapid passage of the barium from the stomach.

Significance of the sphincter. The fluoroscopic studies during early digestion indicated that the whole prepyloric segment is actively involved in the motor activity which regulates the emptying of the chyme. Fractional analysis on dogs whose sphincter had been resected substantiated this fact. In such dogs the meal of meat and water was retained successfully within the stomach so that the usual cycle of chemical changes was observed. However, there were smaller quantities of material retained within the stomach, indicating that the efficiency of the mechanism for the proper retention of the meal had been impaired.

A check by roentgenogram was made on this observation. The usual meal of meat and water was fed to a dog whose sphincter had been resected for about 2.5 cm. After ten minutes 50 cc. of the meal were aspirated and replaced by an equal quantity of barium mixture. A roentgenogram taken fifteen minutes later showed that much larger quantities of chyme passed into the intestine than was noted at this early period of digestion in a dog with the sphincter intact. Although the meal corresponded to that in figure 2, there was more emptying than shown in figure 3. The loss of the sphincter seriously impaired the efficiency of the mechanism which retains the meal within the stomach and yet the prepyloric segment without the sphincter retained a good portion of the meal within the lumen of the stomach.

These observations confirm the idea that emptying of the stomach begins early and continues throughout the whole digestive cycle. Taking three distinctive points in the cycle as isolated points in a gradually progressing phenomenon, roentgenograms indicate that the emptying of the stomach advances at a progressively increasing rate until it is completely empty. This gradual increase in the rate of emptying is accompanied by a progressive disintegration of the meat as measured by the rise in the total acid value and by a gradual reduction of the meal to a fluid state. The faster emptying depends on progressive relaxation of the marked tonic contraction of the pyloric antrum, and reduction of the vigor and frequency of its peristalsis. The sphincter itself seems to fulfill the rôle of the most efficient segment of the antrum. The activity of the whole antrum seems to be as much concerned with the retention of a meal within the stomach until it is digested, as it is with the passage of the products of digestion on into the duodenum.

RESULTS IN THE SECOND SERIES OF OBSERVATIONS. Correlation of these two methods of study on the dog has shown that the progressive rate of emptying of the stomach is accompanied by several constant phenomena: 1, the appearance of free hydrochloric acid closely associated with the terminal period of emptying; 2, progessive disintegration of the meal of meat to an increasingly fluid consistence, until it is reduced finally to a homogeneous viscid fluid state, and 3, changing balance between the raw or partially digested protein of early digestion, and the split products from protein disintegration in later digestion. Further experiments were performed to measure the extent to which any of these factors could determine the motor state of the antrum, and hence the rate of emptying of the stomach.

Acid factor. In a large series of fractional gastric analyses, free acid usually appeared at the beginning of the second phase. Between this point and the preceding aspiration late in the first phase, the stomach had emptied itself of its chyme. The method of study did not give an exact measure of the relationship between the completion of this emptying and the appearance of the acid. However, scattered observations in the course of 275 complete fractional studies (1500 single aspirations) indicated that the appearance of free acid was not an essential factor in bringing about complete emptying of the stomach. A small group of dogs was noted in which considerable free acid was present while the stomach was still distended with a relatively large quantity of fluid chyme. A second group, less frequently noted, was of dogs in which the usual cycle of digestion progressed, the stomach emptied itself, and yet free hydrochloric acid did not appear in the specimen aspirated from the empty stomach early in the second phase. If the presence of free acid controls the emptying process, these two small groups represent a physiologic anomaly.

Careful watch was kept for a dog in the second group which did not show free acid after the stomach had emptied itself in order to make a roentgen-ray observation of the emptying of the stomach in the absence of free acid. In the instances studied the dog was fed the usual meal of meat and water, and the usual aspirations were made during the first phase until the second phase was reached. The first aspiration of the second phase showed that the stomach had successfully emptied itself, yet there was no free acid present in the stomach. Immediately 100 cc. of barium mixture were introduced. A roentgenogram taken fifteen minutes later showed almost complete evacuation of the stomach, as in the average dog, dependent on complete relaxation of the prepyloric segment, with an appearance identical with that shown in figure 4. After the roentgenogram was taken, the barium meal was aspirated. Chemical analysis showed that there was still absence of free acid. So the terminal relaxation was attained and it persisted in these dogs in the absence of free hydrochloric acid.

A second check on the influence of acid toward relaxation of the pyloric

antrum was made by administering a usual meal of meat that had a definite free acid titer. The usual meal of meat and water was acidulated by the addition of free hydrochloric acid, until its free acid value was 16. This is an average value of the juice aspirated after the stomach has emptied itself. It was fed to a dog which had not eaten for thirty-six hours. This longer fast was necessary before the dog would eat the acid meal. A roentgenogram taken after fifteen minutes showed that the free acid could not relax the antrum by overcoming the contractile state established in the presence of the meat as none of the meal passed into the duodenum. The entire meal was aspirated immediately after the roentgenogram was taken, and titration showed that the free acid value was now 9. The presence of this free acid persisted through the fifteen-minute period, yet the vigorous motor activity of the antrum established in response to the stimulus of undigested food persisted despite the presence of the free acid.

The question might be raised whether the action of the free acid on the duodenal side of the pylorus closed the sphincter and prevented the egress of food. Barium mixture with a free acid value of 9 was introduced into a fasting stomach, and a roentgenogram was taken fifteen minutes later. The mixture passed the pylorus readily. This control observation supports the interpretation of the former observation, that the contractile response of the antrum to the stimulus of raw meat predominates over any action that free acid might exert; and the action of the acid in the duodenum did not prevent emptying.

Fluid state. One striking phenomenon in the digestive cycle is the progressive reduction of the meal of meat to its final homogeneous fluid state. Presumably this change in the consistence of the meal might facilitate the passage of the chyme through the pylorus. To measure the influence of the consistence of the meal on emptying, the usual volume of meat was reduced to a fraction of its former volume by dehydration, and it was administered with the usual amount of fluid. This gave a highly fluid meal with a chemically equivalent amount of meat used in all these studies. The usual 85 grams of meat were reduced to a quantity of 30 cc. by drying over a water bath and in an oven. The residue was pulverized and fed to a fasting dog; 255 cc. of barium mixture were introduced with a stomach tube. This method of administration allowed the protein to exert its usual stimulating influence which would have been reduced if suspended diffusely in 255 cc. of barium mixture. A roentgenogram taken after fifteen minutes showed that the meat had again established a state of motor activity in the prepyloric segment which permitted the passage of only a limited quantity of this highly fluid mixture from the stomach corresponding to figure 2.

Products of digestion. Another constant phenomenon observed in using the method of fractional analysis was the disintegration of the raw meat

into intermediary products of digestion as indicated by the rising value of the total acids. To determine whether the products of digestion act in a specific way to stimulate relaxation of the antrum the meat was mixed with the products of late digestion to see if these digestive products could effect passage of the meal from the stomach. The usual meal of meat and water was administered to a dog, and the usual aspirations were made until a late period in the first phase was reached. The whole content of the stomach, 110 cc. of the products of digestion late in the first phase of the cycle, a semifluid mass with a combined acid value of 52, was aspirated. This was used as the fluid vehicle for a test-meal given to another dog. To this partially digested meal, 85 grams of raw meat and sufficient barium mixture to bring the whole volume up to the usual 255 cc. were added; this mixed meal was fed to a fasting dog. A roentgenogram taken after fifteen minutes showed that none of the mixture passed from the stomach. Thus it is seen that the products of late digestion cannot, by any specific stimulus to relaxation, nullify the motor response of the prepyloric segment established in response to the stimulus of raw protein.

COMMENT. In the first series of observations, those made at the three distinctive points in digestion represent isolated periods in a gradually advancing process. They showed that the rate of emptying increased progressively from a slow rate early in the first phase of digestion to a rapid rate late in the first phase. Fluoroscopic studies indicated that the motor activity of the prepyloric segment is probably the chief factor in regulating the rate of emptying. This gradual increase in the rate of emptying is dependent on progressive relaxation of the antrum, and a decrease in the vigor of its peristaltic activity. The motor state of this segment appears wholly independent of that of the fundus. Similarly, Alvarez concluded from his studies that there is "dissociation between the activities of the fundus and the pars pylorica."

Fluoroscopically, the fundus appears as a large motionless ball which contains most of the meal. Peristaltic activity is not marked; simply an irregular wavering is present around its periphery. Alvarez, in studying strips of muscle excised from the fundus, noted that they gave "irregular rounded contractions whose amplitude was small." He continued that the muscle seemed particularly fitted to maintain steady tonic pressure on its contents. In the present studies the fundus became progressively smaller as its contents were diminished, apparently accommodating itself with a type of tonic pressure to the decreasing volume of the meal.

The antrum, on the contrary, was vigorously contracted to a state of "systole," observed by Alvarez (1928), Wheelon (1920, 1922), M'Crea (1924) and others. It was contracted to a small, elongated tube extending from the incisura to the sphincter. Frequent peristaltic waves, often two

to three at a time, traversed this segment. This activity corresponds in a suggestive way to Alvarez's observations with strips of muscles excised from the pyloric antrum. The contractures of these strips when studied by a kymograph made curves of large amplitude, and with sudden rises from the base line. In the present studies the antrum seemed to respond with vigorous tonic and peristaltic activity to the stimulus of the meal, as if it were a more irritable segment than the fundus. Ducceshi observed that the antrum responded more quickly and more energetically to mechanical stimuli than the fundus. It is known that a balloon placed in the pars pylorica will show contractions of that segment, whereas a similar balloon in the fundus will not evoke a similar response. In my studies there appears to be a difference in the irritability of the two segments to the stimulus of undigested meat.

The control of emptying of the stomach cannot reside in the sphincter alone. Resection of the sphincter showed reduction in the efficiency of food retention, but enough food was retained to warrant the conclusion that the whole pars pylorica participates in this control. The sphincter is probably the most efficient segment of the pyloric antrum. From the results of these studies there is no reason to conclude otherwise than that the activity of the sphincter is under the control of the motor activity of the whole phyloric antrum. Wheelon and Thomas, using separate balloons in the antrum and sphincter, demonstrated a coördinated and reciprocal motor activity at the two points. The motor activity in their studies was in response to a constant mechanical stimulus. The present studies have indicated that with a gradual disintegration of the raw protein there is a gradually declining stimulus, so that there is probably a graded and coördinated relaxation of the antrum and sphincter.

In the second part of the studies, different factors observed in the course of digestion were balanced, one against the other, to determine which exerts the greatest influence in altering the motor state of the pars pylorica.

The first factor studied was that of free hydrochloric acid. The most convincing work on the acid control of the pylorus is Cannon's (1911) roentgen studies with various types of foods and his studies with pyloric fistulas, from which he concluded that free acid on the gastric side relaxes the pylorus and on the duodenal side causes its closure. In my studies with fractional gastric analysis progressive emptying during the whole digestive cycle was observed even with the absence of free hydrochloric acid. Further experimental study showed that free acid could not relax the segment in the presence of the stimulus to contraction from raw undigested protein. The usual vigorous motor activity characteristic of early digestion was established despite the presence of free hydrochloric acid, and no part of the meal left the stomach. Furthermore, dogs were ob-

served who completed the digestive cycle and completely emptied the stomach without the appearance of free hydrochloric acid. These results of study on a large series of dogs with the use of fractional test-meals, indicate that the appearance of free hydrochloric acid is probably more an associated phenomenon then a regulatory one. It is dependent, probably, on the persistence of an elevated rate of secretion of free acid, after all the food that can combine with it has passed from the stomach.

It is a widely accepted conception that the more fluid the consistence of a meal, the more rapidly it will empty from the stomach. My studies show, however, that raw protein can establish motor activity in the pyloric antrum which will prevent the rapid egress of an almost wholly fluid meal so that consistence alone does not suffice to control emptying. In the course of normal digestion, however, these two factors do not have the antagonistic relationship of this study. On the contrary, as the fluidity of the meal increases, there is a parallel disintegration of the raw protein to the products of digestion late in the first phase of the cycle. This increased fluidity probably facilitates the passage of the contents of the stomach through the pyloric antrum, which gradually relaxes as the raw protein is disintegrated. In this study there was no evidence to indicate that the products of digestion at this point can act in a specific manner to stimulate relaxation of the pars pylorica.

SUMMARY

The method of study by the combined use of fractional analysis and of roentgenograms has allowed close correlation between the changes in the motor activity of the stomach and the parallel changes in the chemical status of the gastric contents. It has permitted a comparative study of the influence of various factors on the motor state of the stomach so far as these factors might control the mechanism of emptying.

The rate of emptying of the stomach increases progressively from early digestion until the process is complete. It depends on progressive relaxation of the pars pylorica of which the sphincter is the most efficient segment. The mechanism for control does not appear to be a specific stimulus to relaxation of the segment by free hydrochloric acid or the products of digestion. Rather does the activity of the antrum seem to depend on its irritability, and, in these studies, on the stimulating action of raw protein to vigorous tonic and peristaltic contraction. With the gradual disintegration of the protein to the products of digestion late in the first phase of the digestive cycle, there is apparently a graded reduction in the intensity of the stimulus to the antrum and consequently a progressive relaxation of the antrum with more rapid emptying. The parallel change of the meal to a fluid state probably facilitates the emptying of the stomach.

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THE VOLUME OF THE SPLEEN IN TRAUMATIC SHOCK

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It has long been known that the spleen, by virtue of its smooth muscle, can contract and expand (Roy, 1880). Moreover, as long ago as 1854, Gray expressed the view that red blood corpuscles are stored in the spleen. It has been only in recent years, however, that the importance of these functions of the spleen has been appreciated. The work of Barcroft and his collaborators (1923–27) and that of numerous other workers (Hanak and Harkavy, 1924; de Boer and Carrol, 1924; Hargis and Mann, 1925; Scheunert and Krzywanek, 1926; Cruickshank, 1926; Binet and Verne, 1927; Abderhalden and Roske, 1927; Viale, 1927; Izquierdo and Cannon, 1928) has demonstrated beyond any reasonable doubt that an important function of the spleen consists in its ability to serve as a reservoir of widely varying capacity for red blood cells. The blood contained in the spleen has been found to be twice as rich in red corpuscles as is the blood in the rest of the body (Scheunert and Krzywanek, 1926). Stimulation of the sympathetic nerves which run along the splenic vessels causes an immediate and extensive contraction of the musculature of the spleen, resulting in an abrupt diminution in splenic volume coincident with the sudden discharge of its corpuscle-laden blood into the circulation. Izquierdo and Cannon (1928) showed that the polycythemia of emotional states is a manifestation of this neuro-muscular function of the spleen, and pointed out the important protective rôle which this sympathico-splenic mechanism plays in the adjustment of the organism to meet emergencies. From the data available Izquierdo and Cannon calculated that contractions of the spleen may commonly bring about increases of over 10 per cent in the total volume of the circulating blood and of at least 20 per cent in its red blood cell content. In view of these findings it is evident that a study of the behavior of the spleen is pertinent to any physiological or pathological condition which is associated with a change in the volume of the circulating blood.

The work of Keith (1919) and that of Gasser, Erlanger and Meek (1919) demonstrated that there is a decreased volume of circulating blood in

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animals suffering from traumatic shock. In advanced shock the diminution in blood volume is so great that many workers believe that it in itself explains the lowered arterial pressure which characterizes that condition (Cannon, 1923). The question now arises as to whether this decreased blood volume in shock is caused wholly or in part by a dilated spleen. Does the shocked animal bleed to death into its own spleen?

The purpose of this paper is to report some experiments performed to determine the size of the spleen in traumatic shock. Ten experiments were done and all of the ten are included in the report. Cats were used as the subjects of the experiments. Ether anesthesia, administered by tracheal cannula, was used in five of the experiments and amytal anesthesia (0.8 to 1.0 cc. of 10 per cent amytal per kgm.) in the other five. A carotid artery was cannulized and the blood pressure recorded. Shock was produced by combinations of two or more of the following procedures: 1, prolonged ether anesthesia; 2, prolonged amytal anesthesia; 3, crushing of the thigh muscles; 4, crushing of the testicles; 5, hemorrhage; 6, laparotomy; 7, distention of the peritoneal cavity with air. When the blood pressure had fallen to a shock level and had remained there for some time, the spleen was removed and weighed. A résumé of the experiments is given in table 1.

Barcroft, Harris, Orahovats and Weiss (1925) placed metal clips about the edges of the spleen and estimated its range of volume during life from the dimensions shown by x-ray photographs. They found the spleen to be much larger in life than in death. After death it was regularly contracted to approximately its minimum size. The *post mortem* weight in the cat was found to vary in extreme cases from 0.23 to 1.43 per cent of the body weight. In most cases, however, the *post mortem* weight fell between 0.33 and 0.50 per cent of the body weight.

In table 1 it may be seen that in eight cats in various stages of shock the weight of the spleen ranged from 0.18 per cent to 0.35 per cent of the body weight. The average of the 8 determinations was 0.25 per cent. The *post mortem* weight of the spleen in the two cats that died in shock was 0.26 per cent of the body weight in one case and 0.21 per cent in the other. Thus in all ten experiments the spleen's weight corresponded to the lowest *post mortem* weights reported by Barcroft and his associates, values much lower than were found to be the case during life. This evidence seems to indicate that there occurs an intense constriction of the spleen in shock. The appearance of the spleens removed from the shocked animals corresponded with this interpretation. Without exception they were small, firm and dry. When the vessels at the hilus were divided there was no bleeding from their splenic stumps. If the spleens were cut across with a knife there was no hemorrhage from the cut surfaces. In other words, the

organs were practically bloodless. It is quite evident, then, that the shocked animal does not lose its blood into a dilated spleen.

TABLE 1

EXPERIMENT NUMBER	WEIGHT OF CAT	ANESTHESIA	SHOCKING PROCEDURES OTHER THAN ANESTHESIA	HOUR AT WHICH SPLEEN WAS REMOVED	SYSTOLIC BLOOD PRESSURE DURING HOUR PRECEDING SPLENECTOMY	WEIGHT OF SPLEEN		REMARKS
						In grams	In per cent of body weight	
	kgm.				*mm.* Hg		*per cent*	
1	2.8	Ether	Crushing of thigh muscles and of testicles	6th hr.	50–45	8.0	0.29	
2	3.3	Ether	Crushing of thigh muscles and of testicles	5th hr.	72–62	9.0	0.27	
3	3.9	Ether	Crushing of thigh muscles and of testicles	6th hr.	105–88	7.2	0.18	
4	3.75	Ether	Crushing of thigh muscles and of testicles	3rd hr.	75–55	7.2	0.19	Aged animal
5	3.6	Ether	Crushing of thigh muscles and of testicles	End of 2nd hr.	90–60	10.0	0.28	
6	3.3	Amytal	Laparotomy-peritoneum inflated with air to 20 cm. H_2O pressure for 2 hrs.	8th hr.	75–70	11.6	0.35	
7	1.6	Amytal	Exposure to cold	9th hr.	95–80	3.2	0.20	
8	1.95	Amytal	Laparotomy-peritoneum inflated with air to 40 cm. H_2O pressure for 45 min.	During 4th hr.	120–0	5.0	0.26	Died few minutes before spleen removed
9	2.2	Amytal	Laparotomy-peritoneum inflated with air to 40 cm. H_2O pressure for 2 hrs. —hemorrhage	8th hr.	80–60	5.7	0.26	
10	3.5	Amytal	Crushing of thigh muscles and of testicles	9th hr.	95–0	7.2	0.21	Died few minutes before spleen removed

The presence of a markedly constricted spleen in shocked animals seems significant for the behavior of the splanchnic vasomotor mechanism in shock. Present knowledge indicates that the speen may be considered as a large and modified blood vessel and that its constriction and relaxation

are subject to the same influences that modify the tone of blood vessels in general. The smooth muscle of the spleen appears to be under the same sympathetic control as the blood vessels of the splanchnic field. The spleen has been found to be contracted in asphyxia, in exercise, in hemorrhage, in emotional excitement, and in death from carbon-monoxide poisoning (Hanak and Harkavy, 1924; de Boer and Carrol, 1924; Barcroft, Harris, Orahovats and Weiss, 1925; Scheunert and Krzywanek, 1926; Abderhalden and Roske, 1927; Binet and Verne, 1927; Izquierdo and Cannon, 1928), all of which are conditions associated with a constriction of the splanchnic vessels in general. It is dilated in rest (Barcroft, Harris, Orahovats and Weiss, 1925) and it is relaxed periodically during digestion (Hargs and Mann, 1925); under similar circumstances the splanchnic vascular bed as a whole shows the same variations. Presumably, then, the volume of the spleen can be taken as an index of the state of contraction of the splanchnic blood vessels.

In 1903 Crile reported a large series of experiments from which he concluded that the low blood pressure which characterizes shock is the result of an exhaustion of the reflex vasoconstrictor mechanism. Later Mummery (1905) reported observations which he interpreted as supporting the same conclusion. Since the early report of Crile, however, a great amount of evidence has been presented which seems to show conclusively that vasoconstrictor paralysis, when it occurs in shock at all, appears only in the terminal stages, when it may arise from asphyxia of the medullary centers and is the result of the low blood pressure and not the cause (Porter, 1907; Henderson, 1908; Malcolm, 1909; Seelig and Lyon, 1909, 1910; Mann, 1914; Seelig and Joseph, 1914; Morison and Hooker, 1915; Muns, 1915; Guthrie, 1917; Cattell, 1922; Wallace, Fraser and Drummond, 1917; Erlanger, Gesell and Gasser, 1919; Cannon, Fraser and Hooper, 1919; Cannon, 1923). One still hears, however, frequent reference to vasoconstrictor paralysis as a causative factor in shock.

In the experiments reported in this paper the spleen was found to be extremely constricted in cats in various stages of shock. The close relationship which exists between the state of contraction of the spleen and that of the splanchnic vessels in general seems to justify the conclusion that in the experiments reported the splanchnic vasoconstrictor mechanism was operating powerfully and efficiently at a time when the blood pressure was below the critical level. Such evidence seems to point unconditionally to a determined, though often ineffective, activity of the vasoconstrictor system in traumatic shock in an attempt to maintain the normal arterial pressure in the face of a rapidly decreasing blood volume. This conclusion is in agreement with the findings of many other investigators who have studied this problem.

Recently it has been suggested (Journal American Medical Association,

1929, xcii, 987, Current Comment) that the frequent failure of adrenin to restore normal blood pressure in clinical cases of shock points strongly toward the existence of a vasoconstrictor paralysis. There is another interpretation of this inefficacy of adrenin in shock which is more in keeping with the evidence at hand. The usual pressor response to adrenin is the function of a vasoconstrictor system which has been, immediately before the injection, only moderately active. The widespread stimulation of the sympathetic endings by adrenin throws into action every peripheral component of the entire system. The natural consequence is a marked rise in blood pressure. On the other hand, the evidence presented in this paper together with the observations of many other investigators indicates clearly that in shock the vasoconstrictor mechanism is exerting itself to its utmost in the attempt to maintain an efficient head of pressure in the presence of a progressively diminishing blood volume. There is no reason to believe that any part of the system is idle. Then is it not to be expected that the administration of adrenin in shock will be followed by a much slighter rise in blood pressure than is caused by that drug when it is given in normal conditions?

A similar interpretation may be applied to the recent work of Smith (1926) who found that shock increased the susceptibility of the peripheral vasoconstrictor mechanism to the depressive action of ergotamine and to adrenin "reversal." Smith interpreted these findings as indicating that in shock there is a tendency for inhibitor vascular responses to predominate over the usual augmentor responses. It seems to the writer that a more reasonable interpretation may be made on the basis of the heightened activity of the vasoconstrictor mechanism in shock. Since in shock every sympathetic ending is under constant central activation, then the slightest depressor effect of ergotamine or of adrenin "reversal" will be reflected in the blood-pressure curve, whereas in the normal animal the existence of only a moderate vascular tone may limit these effects and prevent them from markedly influencing the blood-pressure level.

In generalizing upon the presence of splanchnic vasoconstriction in traumatic shock one reservation is necessary. Exposure or manipulation of the abdominal viscera rapidly leads to local congestion. This is a purely local response to trauma, residing probably in the muscle of the vessel wall, and is not due to a paralysis of the vasoconstrictor center (Porter, Marks and Swift, 1907; Cannon and Murphy, 1907; Henderson, 1909). In cases of shock occurring after a laparotomy and associated with prolonged exposure and trauma of the abdominal viscera this inhibition of the vascular musculature may be maintained for some time in spite of the efforts of the sympathetic nerves to restore contraction. Such a congestion may complicate the picture of shock in many instances, but it is a special feature and is no more typical of shock states in general than is the

embolism which may complicate a shock-producing fracture of the femur or the local intracranial injury which may accompany a fracture of the skull.

SUMMARY

Ten experiments to determine the state of contraction of the spleen in cats in various stages of traumatic shock are reported. In every instance the spleen was found to be so extremely constricted that its cut surface did not bleed, and to have a weight ranging from 0.18 per cent to 0.35 per cent of the body weight and averaging 0.25 per cent. These weights are similar to the lowest *post mortem* values obtained by Barcroft, Harris, Orahovats and Weiss (1925) in cats, and correspond to the minimum weight of the completely contracted spleen. Evidently, then, a dilated spleen is not a factor in the production of the decreased blood volume which is characteristic of traumatic shock.

The close relationship between the contracted state of the spleen and the contracted state of the splanchnic vascular bed is discussed, and the conclusion is reached that, as regards its smooth muscle, the spleen may be looked upon as a large and modified splanchnic blood vessel the volume of which serves as an index of the state of contraction of the splanchnic vascular bed as a whole. The occurrence of an extreme constriction of the spleen in traumatic shock is interpreted as supporting the observations of other investigators to the effect that the splanchnic vasoconstrictor mechanism becomes highly active in shock in an attempt to maintain an effective blood pressure in the presence of a rapidly diminishing blood volume.

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THE EFFECTS OF CERTAIN FOODS UPON THE RATE OF THE DENERVATED HEART OF THE SURVIVING UNANESTHETIZED CAT

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For several years Cannon and his associates have made a study of the behavior of the mammalian heart after elimination of its central nervous control. After the cardiac nerves of the cat have been severed, the resting animal exhibits a heart rate which is slow relative to that of the normal cat. Coincident with the slightest excitement, however, there appear large increments of acceleration which can be abolished by denervation of those glands which, under nervous excitation, are known to secrete cardio-accelerator principles. Thus, after section of the nerves to the adrenal glands and to the liver, the denervated heart of the surviving, unanesthetized cat beats at a slow and steady rate when the animal is at rest, and this rate is not significantly altered by extreme emotional excitement or by periods of very active muscular exertion (Cannon, Lewis and Britton, 1926). The heart of such an animal, during a period in which the body temperature remains constant, serves as a sensitive indicator of any cardio-accelerator or cardio-inhibitor substances which may circulate in the blood stream.

Early in the study of the factors which operate to modify the heart rate of animals so prepared, it was noted that the heart beats at a slower rate when the animal is fasting than when the animal has recently ingested food. Attempts were made by Bard (in 1926) and by MacFall (in 1927) to study this phenomenon, but in both instances the study was discontinued because of the failure at that time to complete a lasting preparation of the denervated heart. Since the further development of the knowledge of the innervation of the heart of the cat, and the consequent improvements in the technique of denervation, it has now become possible to prepare animals whose hearts are so completely denervated that for a number of months after operation the heart rate remains indifferent to any form of emotional excitement or of musculo-skeletal activity. In these animals a study has been made of the effects of the ingestion of vari-

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ous types of foodstuffs upon the heart rate, with the results which are to be reported in this paper.

Preparation of animals. Young adult male cats were used in the experiments to be described. They were subjected to a series of neurectomies aimed to destroy all communication between the central nervous system and the heart and also between the central nervous system and the adrenal glands and the liver. The procedure by which this is accomplished has been described in full in other communications from this laboratory (Cannon, Lewis and Britton, 1926). In the preparation of the animals used in the present study, although from time to time the operative technique was varied in some of its details, the typical method employed consisted in a two-stage operative procedure at each stage of which a unilateral attack was made upon both sympathetic and parasympathetic nerves. With the completion of the procedure the animal was left still possessing its right recurrent laryngeal nerve (which has no cardiac branches) to supply the vocal cords, and its left vagus trunk (with cardiac branches severed) to supply the motor and secretory functions of the gastro-intestinal tract. All vagal fibers to the heart had been severed. Both sympathetic trunks had been removed from above the stellate ganglia to the level of the pelvic brim (Cannon, Newton, Bright, Menkin and Moore, 1929), i.e., throughout the entire range within which they communicate with the central nervous system, and all sympathetic endings in the heart, adrenal glands and liver (in fact within the entire body) had thus been isolated from central nervous or reflex nervous control. With the application of proper precautions to prevent the occurrence of sepsis and unnecessary trauma, and with diligent post-operative care, the animals survived the procedure surprisingly well, and within a week were eating their normal diet heartily and gaining weight.

The performance of this procedure is not considered in itself conclusive proof of the denervation of the heart. It is easy to miss certain of the cardiac branches of the vagus nerves and some of the animals have given evidence of failure of denervation by displaying reflex cardiac acceleration of considerable magnitude after the denervation was supposedly complete. Each animal, therefore, was repeatedly tested throughout its period of use for evidence of nervous influence on the heart rate, and whenever evidence of this nature was obtained the animal was immediately discarded. The method used for the testing was that of emotional excitement combined with muscular activity. Although at the start most cats will react very energetically when approached by a barking dog, they soon learn not to fear the average dog, or the dog learns to fear the cat. A more reliable method has been found to consist in the utilization of the cat's antipathy to being fastened upon its back upon an animal board. The usual procedure was as follows. The cat is easily trained to lie quietly on a

pillow on the observer's lap. While the animal rests or dozes in this fashion, its heart rate is recorded by means of a tambour held on the chest wall over the cardiac impulse and connected through a rubber tube with a recording tambour which writes on a kymograph just above a five-second time record. The heart rate is followed for five minutes, or as much longer as is required to secure the true basal or resting rate after the animal has remained absolutely quiet for several minutes. After the basal rate has been determined the cat is fastened to an animal board by its neck and two front legs. Usually no teasing is required to incite the animal to fly into a fury, straining at its leashes, vigorously thrashing about with its hind quarters, lashing its tail, howling, spitting and snarling. One or two minutes of such vigorous display usually suffices temporarily to exhaust the animal and it then lies panting while the observer again records its heart rate. The struggle can be repeated enough times to determine accurately the presence or absence of an increased frequency of the heart beat relative to the rate after five minutes of quiet rest in the lap. In cats with thick or deformed chest walls it is sometimes necessary to substitute stethoscope readings for the graphic records.

None of the cats used in the experiments to be recorded in this study showed under the conditions described above an acceleration which exceeded ten beats per minute (the normal cat gives an acceleration of over 100 beats per minute under similar circumstances). This slight increment does not necessarily point to the persistence of nervous connections to the heart. It can be reasonably explained on the basis of change to the dorsal posture, the rise in body temperature which accompanies such extreme exertion, or mechanical effects of increased thoracic respiratory excursion. Moreover, such animals, under varying conditions of less severity, exhibit a constant heart rate with no respiratory arrhythmia, whereas animals possessing known nervous remainders show a changing heart rate under quiet conditions, a more marked acceleration upon struggling and respiratory arrhythmia.

EXPERIMENTAL METHOD. At the very beginning of the study it was noted that the ingestion of a mixed meal was followed by an acceleration of the beat of the denervated heart. The problem then became one of a careful record of the changes in heart rate following the ingestion of foods. From preliminary experiments it appeared that an 18 to 20 hour period was required for the heart rate to return to its fasting level after a large meal, and that once this level was reached there was no significant change in the rate for several hours. Consequently it was made a rule not to administer the food, the effects of which were to be studied, until 18 to 24 hours after the last feeding. In each instance the fasting heart rate was determined by two or three hourly readings; then the animal was fed and by hourly or half-hourly readings the effects of the feeding upon the rate were deter-

mined. Each reading throughout the entire experiment was made while the animal lay quietly on a pillow in the observer's lap. The regular procedure was to count the heart rate for five successive minutes, using a stethoscope and a stop-watch, and to consider the lowest of the five readings to be the resting heart rate at that particular time. The following is a sample protocol of the type of experiment to be reported:

Cat 384. October 23, 1928. Fasting 22 hours at 7:00 a.m.

Heart rates:

7:05 a.m.—92; 7:06—92; shook cat vigorously—7:07—97; 7:08—95; 7:09—96. (basal rate—92)
7:59 a.m.—91; 8:00—92; 8:01—93; 8:02—92; 8:03—92. (basal rate—91)
8:53 a.m.—92; 8:54—92; 8:55—94; 8:56—94; 8:57—94. (basal rate—92)
9:00—9:40 a.m.—ate 145 grams ground lean beef
9:50 a.m.—106; 9:51—107; 9:53—109; 9:54—108; 9:55—108. (basal rate—106)
10:31 a.m.—105; 10:32—104; 10:33—104; 10:34—105; 10:35—107; 10:36—108. (basal rate—104)
11:30 a.m.—112; 11:31—115; 11:32—115; 11:33—116; 11:34—116. (basal rate—112)
11:55 a.m.—114; 11:56—114; 11:57—115; 11:58—115; 11:59—114. (basal rate—114)
12:27 p.m.—112; 12:30—110; 12:31—111; 12:32—111; 12:33—111. (basal rate—110)
1:28 p.m.—106; 1:29—106; 1:30—106; 1:31—109; 1:32—111. (basal rate—106)
2:31 p.m.—106; 2:32—108; 2:33—110; 2:34—111; 2:35—110. (basal rate—106)
3:12 p.m.—107; 3:13—107; 3:14—109; 3:15—109; 3:16—109. (basal rate—107)
3:50 p.m.—108; 3:51—110; 3:52—109; 3:53—108; 3:54—109. (basal rate—108)
5:17 p.m.—107; 5:18—109; 5:19—108; 5:20—110. (basal rate—107)
6:23 p.m.—108; 6:24—107; 6:26—108; 6:27—108; 6:28—109. (basal rate—107)
8:03 p.m.—105; 8:04—106; 8:05—109; 8:06—108; 8:07—109. (basal rate—105)
8:59 p.m.—105; 9:00—106; 9:01—106; 9:02—106; 9:03—107. (basal rate—105)
10:06 p.m.—104; 10:07—106; 10:08—106; 10:09—108; 10:10—108. (basal rate—104)
11:04 p.m.—102; 11:05—100; 11:06—102; 11:07—101; 11:08—101. (basal rate—100)
12:10 a.m. October 24—102; 12:11—102; 12:12—105; 12:13—105; 12:14—104. (basal rate—102)
1:50 a.m.—97; 1:51—99; 1:52—98; 1:53—97; (basal rate—97)
3:55 a.m.—90; 3:56—92; 3:57—92; 3:58—90; 3:59—91. (basal rate—90)
5:34 a.m.—93; 5:35—94; 5:36—94; (basal rate—93)
7:34 a.m.—91; 8:35—91; 7:36—92; (basal rate—91)

As stated above, the animals used in this study were young male cats whose hearts were so completely denervated as to exhibit an acceleration in rate of not more than ten beats per minute when the animal passed from a state

of bodily rest to one of violent muscular exertion combined with extreme emotional excitement. This variation of ten beats per minute, then, represented the maximum error in the heart-rate readings in such an animal, but, since in the course of experiments of the type here recorded the tamed animal needed never to become even moderately excited, the actual margin of error was really below this figure. Cat 384 was the subject of the experiment described in the protocol given above and was representative of the group used. One may see that in the absence of excitement and

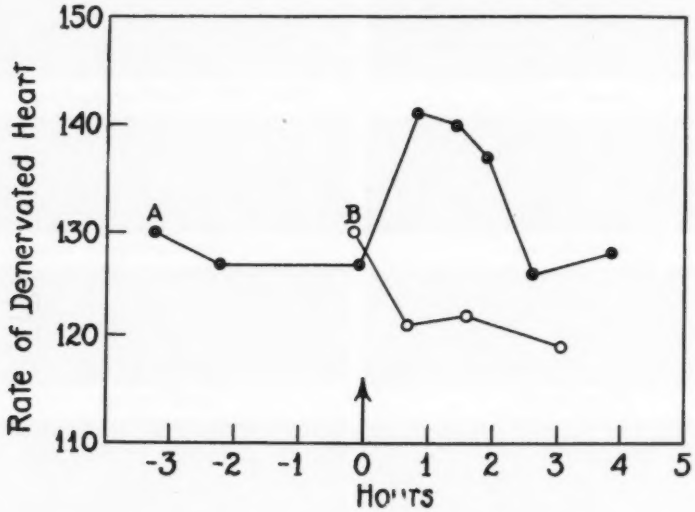

Fig. 1. Changes in rate of the denervated heart from the basal level when cat 336 was given liquids. Curve A shows the acceleration caused by 90 cc. of whole milk; curve B, the absence of effect after 50 cc. of water. The arrow marks when liquids were given. The readings prior to that time serve as controls. The length of the arrow represents the maximum acceleration of the heart after violent struggle.

activity the maximum and minimum minute rates during a five-minute period of rest in the lap never differed by more than five beats. Furthermore, the first of the five successive readings was more frequently the slowest than it was the fastest rate of the five, so evidently the disturbance caused by lifting the cat into the lap at the beginning of the five-minute period did not cause an appreciable acceleration. Repeated experiments of the type shown above have led to the conclusion that the series of readings obtained in this manner may be considered as reasonably representative of the changes in the resting rate of the heart throughout the period of the experiment, and it has been through this type of experiment that the

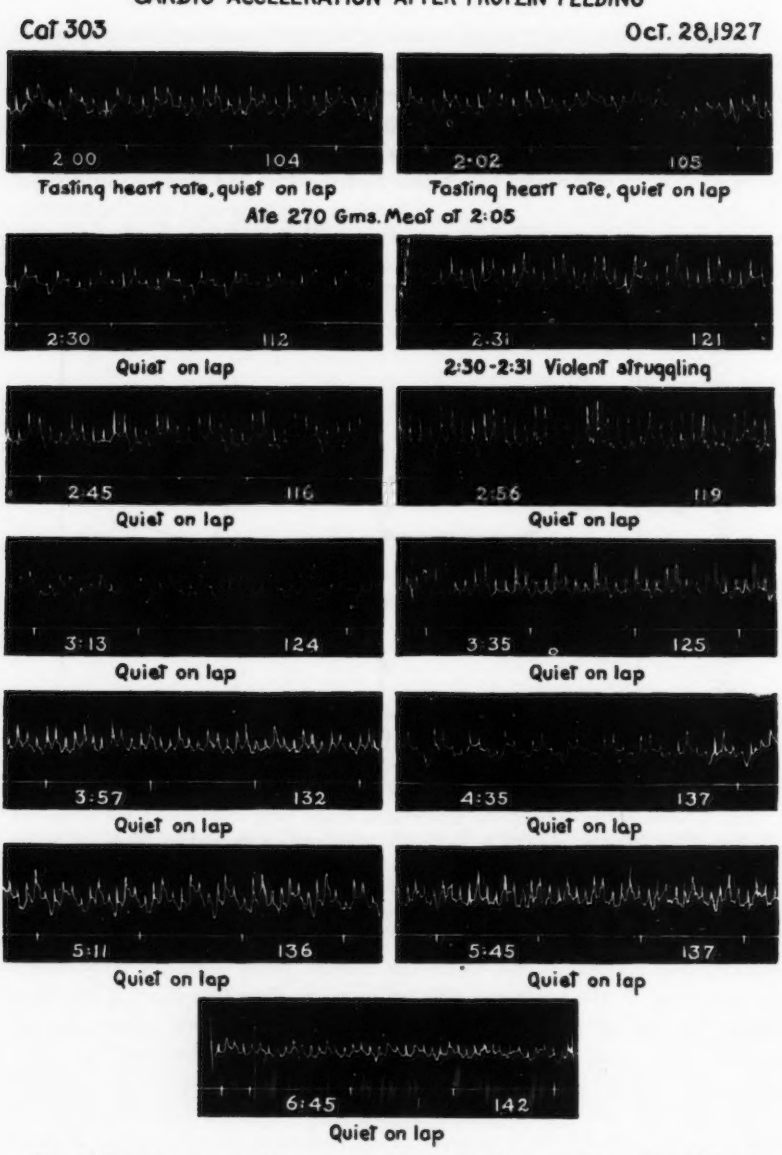

Fig. 2. Graphic record of the acceleration of the denervated heart (cat 303) after a meal of meat. The heart was accelerated only 9 beats per minute by violent struggle (2:30–2:31); the protein meal caused a progressive increase in heart rate amounting, at four hours, to an acceleration of 37 beats per minute above the fasting level.

results recorded below have been secured. Whatever slight error excitement introduces into such a series of readings tends to minimize the effect of the feeding rather than to exaggerate it, for the animal which is fasting wanders about crying for food, whereas during the hours just after a meal it lies quietly dozing.

RESULTS. In figure 1 it may be seen that following the administration of a non-nutritive liquid such as water to a cat whose heart is denervated there occurs no rise in the rate of the heart beat, but in fact in this particular case a fall. On the other hand, when a nutritive liquid such as milk is administered there follows a distinct and measurable increase in the rate which may persist for over two hours. If this effect were due to the absorption of one of the foodstuffs contained in milk it should follow the ingestion of other foods as well. Whole milk contains protein, fat and carbohydrate in moderate amounts and any one or all of these three classes of substances might be responsible for the effects observed. Repeated experiments have revealed that each of these substances when ingested in appropriate quantity may determine an acceleration of the denervated heart, but that the extent to which they are endowed with cardio-accelerator properties varies in a highly interesting and suggestive fashion.

When a moderate or even a small meal of a protein food, such as lean beef, is fed there invariably appears a cardio-acceleration of surprising magnitude and duration. In figure 2 may be seen graphic records of the frequency of the cardiac impulses in cat 303, taken before and after the ingestion of a large meal of meat. Although violent struggling failed to elevate the heart rate more than 9 beats per minute, the protein meal caused a progressive acceleration which reached a maximum of 37 beats per minute—a 35 per cent rise over the fasting level—at 4 hours after the feeding. Figures 3a and 3b show typical heart-rate curves of four different cats before and after their voluntary ingestion of quantities of lean beef varying from 85 grams to 150 grams and containing approximately 22 per cent, or from 19 to 33 grams, of protein and 3 per cent, or from 2.5 to 4.5 grams, of fat (Joslin). These quantities of meat may be considered as constituting small to moderate meals for a cat of 3 kgm. since the normal cat of this size will often eat a considerably larger quantity at one sitting. From figures 3a and 3b it may be seen that such a quantity of protein food caused in these particular animals cardio-accelerations varying from 22 to 30 beats per minute, or an increase amounting to 24 to 36 per cent over the fasting heart-rate level. The accelerations were of such duration that the rate did not return to the fasting level until from 14 to 19 hours after the feeding. In these five curves the peaks of 24 to 36 per cent increase occurred at from 2 to 3 hours after the feeding except in the second curve on cat 384, where the maximum acceleration of 23 beats per minute, a 25 per cent increase, was not reached until the sixth hour. One

can better appreciate the magnitude of these effects by computing the total increase in number of beats for each curve (i.e., the sum of the increases per minute over the fasting level at each hour multiplied by 60). Such a

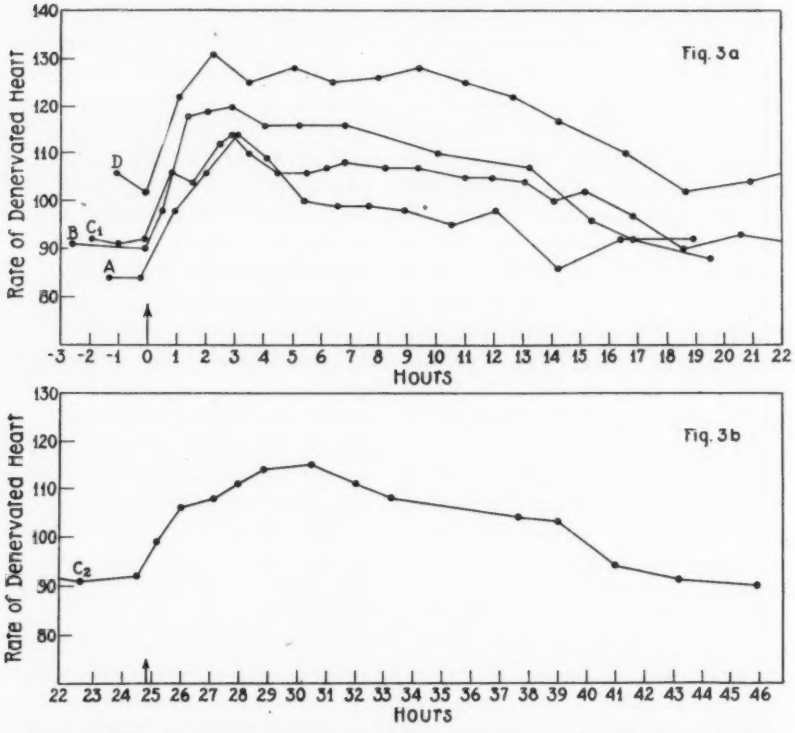

Fig. 3a. Effect of a protein meal (lean beef) upon the rate of the denervated heart in four cats. *A*, cat 403, 85 grams; *B*, cat 411, 150 grams; C_1, cat 384, 145 grams; *D*, cat 400, 145 grams. In each case the beef was eaten at the time marked by the arrow. The length of the arrow represents the average maximum acceleration of the heart in these four cats due to violent struggle. These meals caused accelerations of the denervated heart in the several cats ranging from 13,400 to 22,400 extra beats.

Fig. 3b. Curve C_2 is a direct continuation of C_1 in figure 3a, the two curves showing the rate of the denervated heart in cat 384 throughout a 45-hour period. In C_1 the heart rate returned to the fasting level during the eighteenth hour after the feeding and remained at that level until, during the twenty-fourth hour (C_2), a second meal (130 grams lean beef) was given.

calculation shows that the protein meals, the effects of which are shown in figures 3a and 3b, caused accelerations ranging from 13,400 to 22,400 extra beats in the respective cases. At the rate of 90 beats per minute a heart

beats 13,500 times in 2.5 hours or 16,200 times in 3 hours. In other words, the protein meal threw an extra burden of work upon the heart which, providing other factors than rate remained constant, was comparable in extent to the heart's total performance for 3 hours under fasting conditions.

Lean beef is not peculiar among proteins in this regard. All other meats tried produced like effects. The curves shown are entirely representative of the changes found to occur after meat ingestion in every animal in which the experiment has been performed. For example, liver gives as great an

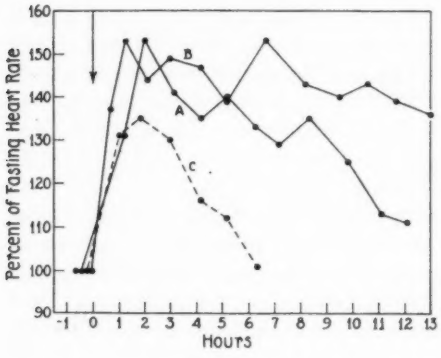

Fig. 4

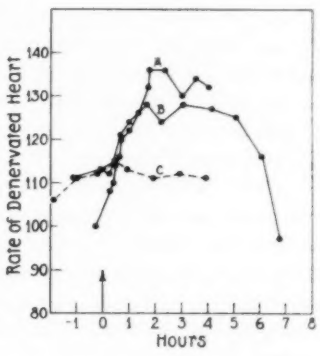

Fig. 5

Fig. 4. Contrast between effects of carbohydrate and protein foods upon the rate of the denervated heart in cat 337. The changes are plotted as percentage increases above the fasting heart-rate level. The feedings were given at the time marked by the arrow. *A*, February 18, 1928, 100 grams beef liver; *B*, February 19, 1928, 100 grams lean beef; *C*, February 21, 1928, 94 grams (mashed) potato.

Fig. 5. The rate of the denervated heart of cat 303 after various meals. Whereas 250 grams of a meat mixture, *A*, or 100 grams of liver, *B*, caused large increments in heart rate, 120 grams of mashed potato, *C*, produced no measurable acceleration. The arrow marks the time at which the meals were given. The length of the arrow represents the maximum acceleration of the heart of this cat caused by violent struggle.

effect as does lean beef (fig. 11), and canned salmon, although hardly a representative protein food, inasmuch as it contains also large quantities of fats, brings about an even greater acceleration at times (figs. 6 and 10).

From a study of figures 2, 3a and 3b and the protein curves in the succeeding figures it seems obvious that a high protein diet is incompatible with cardiac rest. Moreover, since the cat's cardio-acceleration following protein feeding lasts some 15 to 18 hours, it is evident that if the cat eats heartily twice daily its heart will be under constant stimulation throughout the 24 hours and will be beating at an elevated rate at all hours of the day and night. Indeed, a cat which spends its existence amid an environ-

ment of plenty might never in its lifetime be entirely free from this cardio-accelerator action of protein.

In a number of animals a study has been made of the effects of carbohydrate feeding upon the rate of the denervated heart and sufficient evidence has finally been acquired to evaluate the carbohydrate effect relative to that of protein. A great deal of difficulty was encountered in finding a high carbohydrate food which the cat would eat. Of a number of animals tried cat 337 was the only one that would eat mashed potato voluntarily. Figure 4 shows the effect of a meal of mashed potato upon its heart rate. It was then found that cats which would not voluntarily eat mashed potato in any quantity, even when flavored with Liebig's Meat Extract, could often be induced to swallow it when balls of the cooked potato were placed in the back of the mouth. Cats 303 and 336 were fed potato and cornstarch pudding in this manner and figures 5 and 6 show the effect of these carbohydrates upon their heart rate, along with protein curves in the same animals. This method of forced feeding, however, was found to be both laborious and time-consuming, and it introduced into the experiments the undesirable factor of prolonged excitement. Consequently it was abandoned for the quicker and simpler method of gavage. Attempts to give glucose by a stomach tube were disappointing, for even in dilute solution sugar tended to exert a marked cathartic action and was invariably being passed by rectum within an hour. It was then found that the cereal, "Cream of Wheat," the dry weight of which consists of approximately 10 per cent protein and 75 per cent carbohydrate, would not exert this physicking effect if given in only moderate quantities. In the later experiments it has been given, after cooking, as a thick gruel, 100 grams of which can be forced down a tube into the stomach by means of a gastric pump. After receiving a meal of carbohydrate in such manner, the cat which was previously crying for food seems to become entirely contented, and lies quietly purring and dozing in a typical post-prandial fashion. This has led to the assumption that the brief period of discomfort incident to the administration of the food does not notably inhibit digestive secretion and motility, and that the food thus given is promptly digested. Figures 7 and 8 show the contrast in four animals between the effect of "Cream of Wheat" and that of high protein foods.

Through these various methods rather large quantities of carbohydrate food have been introduced into seven cats with denervated hearts. In no instance did the carbohydrate produce an acceleration of the heart at all approaching that produced by protein. The potato effect in cat 337, shown in figure 4, is the greatest carbohydrate acceleration I have ever observed. In this instance 94 grams of mashed potato caused an increase of 35 per cent in the heart rate within 2 hours but the rate had returned to normal before 7 hours, whereas about the same amount of beef caused

an acceleration of over 50 per cent in 2 hours and at 12 hours the rate was still elevated to nearly 40 per cent above the fasting level. The curves in figures 4 to 8 inclusive represent carbohydrate effects. One sees a marked variation among the animals. There was practically an absence of effect in cats 303, 403 and 404, whereas cats 336, 411 and 384 showed small but measurable accelerations from carbohydrate, and cat 337 showed, among

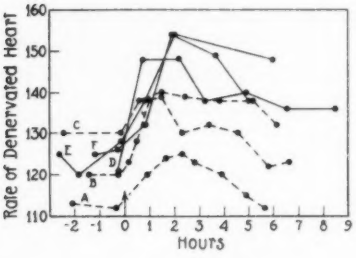

Fig. 6

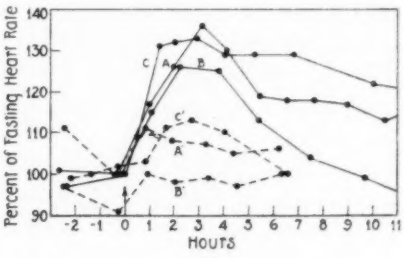

Fig. 7

Fig. 6. Comparison of carbohydrate and protein in their effects upon the rate of the denervated heart in cat 336. All feedings were given at the time marked by the arrow. The length of the arrow represents the maximum acceleration due to violent struggle.

Carbohydrate curves: *A*, 20 grams cornstarch (20 grams carbohydrate); *B*, 96 grams potato (about 17 grams carbohydrate); *C*, 100 grams potato (about 18 grams carbohydrate).

Protein curves: D, 70 grams salmon (15 grams protein and 8 grams fat); *E*, 80 grams liver (16 grams protein); *F*, 156 grams salmon (33 grams protein and 19 grams fat).

Fig. 7. Contrast between the effects of protein and carbohydrate food upon the rate of the denervated heart in three cats. The heart rate is expressed in per cent of the fasting rate. The foods were administered at the time marked by the arrow. The length of the arrow represents the average maximum acceleration of the heart in these three cats due to violent struggle.

Cat 403: *A*, 85 grams lean beef (19 grams protein); *A'*, 20 grams "Cream of Wheat" (15 grams carbohydrate).

Cat 404: *B*, small amount salmon (about 15 grams protein and 8 grams fat); *B'*, 20 grams "Cream of Wheat" (15 grams carbohydrate).

Cat 411: *C*, 150 grams lean beef (33 grams protein); *C'*, 30 grams "Cream of Wheat" (22.5 grams carbohydrate).

several trials, one marked carbohydrate effect, which, however, still did not approach the magnitude of response invariably evoked by protein.

In cat 403, a very tame animal in which carbohydrate produced practically no effect when given orally, the effect of prolonged intravenous administration of glucose upon the rate of the denervated heart was determined. For this purpose the injection apparatus which has been developed in this laboratory by A. R. Colwell, and which permits intravenous injec-

tions at a slow and quite constant rate, was used. For one hour 24 per cent glucose was given intravenously to the unanesthetized cat at the rate of 10 cc. (2.4 grams of glucose) per hour without the heart beat being changed from its fasting rate. Later in the day glucose was again injected for one hour, this time the injection rate being trebled so as to deliver 7.2 grams of glucose within the hour. During the last 15 minutes of the hour the heart beat underwent an acceleration from the previous rate of 105 per minute to 115, although the animal remained quiet. A sample of blood taken during this period of acceleration showed a blood-sugar content of 476 mgm. per cent. This glucose concentration of ap-

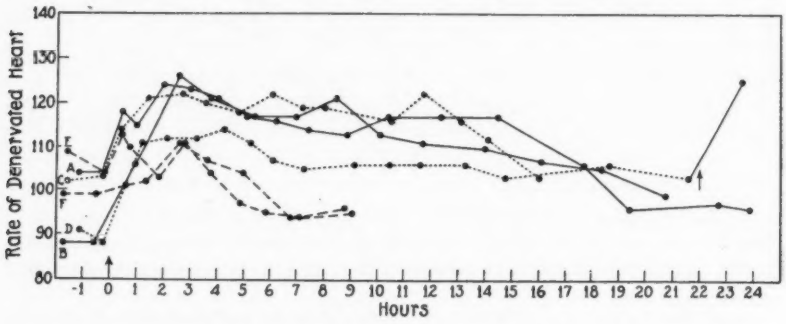

Fig. 8. Comparison of the cardio-accelerator power of the various foodstuffs in cat 384. The feedings were given at the time marked by the arrow. The length of the arrow represents the maximum acceleration due to violent struggle.

Protein meals: *A*, 150 grams lean beef (34 grams protein and 3.5 grams fat); *B*, 150 grams liver (30 grams protein, 4.5 grams fat and 4 grams carbohydrate).

Fat meals: *C*, 35 grams butter (30 grams fat); *D*, 26 grams beef fat (26 grams fat), followed, at 22 hours (arrow) by a small meal of meat.

Carbohydrate meals: *E*, 20 grams "Cream of Wheat" (15 grams carbohydrate and 2 grams protein); *F*, 20 grams "Cream of Wheat" (as above).

proximately 0.5 per cent is greater than can be produced by oral administration of carbohydrate in the non-diabetic animal, and would necessarily exert an osmotic action. It seems reasonable, therefore, to attribute the slight cardiac acceleration to some influence of altered osmotic pressure in the blood, rather than to a specific cardio-accelerator property of the glucose.

Experiments to determine the effects of fat ingestion upon the denervated heart rate have been more recently undertaken. Although fewer observations have been made upon fats than upon carbohydrates and proteins, the experience so far would indicate that fat foods occupy a place midway between carbohydrates and proteins in their cardio-accelerator properties. Figures 8, 9 and 10 show the comparative effects of fat and

protein in three different animals. In cat 336 (fig. 9) the meals of beef fat and olive oil, containing 23 grams and 50 grams of fat respectively, failed to elevate the heart rate to anywhere near the level to which it was forced after meals containing 15 to 16 grams of protein. But in cat 384 (fig. 8) the greater of two responses to fat meals corresponded rather closely in extent and duration to the response to similar quantities of protein. Again in cat 411 fat produced an acceleration which closely approximated that of protein, whereas in cat 344 (fig. 10) a meal of 50 grams

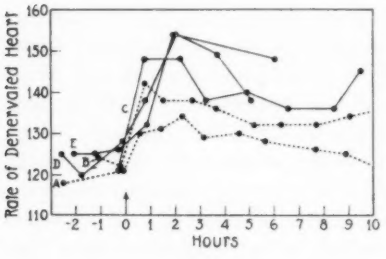

Fig. 9

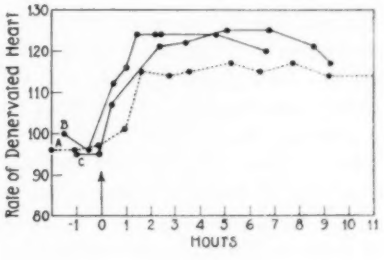

Fig. 10

Fig. 9. Contrast between the effects of fat and protein foods upon the rate of the denervated heart in cat 336. The arrow marks the time at which the feedings were given. The length of the arrow represents the maximum acceleration of the heart due to violent struggle.

Fat meals: *A*, 25 grams beef fat (25 grams fat); *B*, 50 grams olive oil (50 grams fat).

Protein meals: *C*, 70 grams salmon (15 grams protein and 8 grams fat); *D*, 80 grams liver (16 grams protein, 2.5 grams fat and 2 grams carbohydrate); *E*, 156 grams salmon (33 grams protein and 19 grams fat).

Fig. 10. Rate of the denervated heart of cat 344, arrow marking the time at which meals were given. The length of the arrow represents the maximum acceleration of the heart due to violent struggle. Meal *A*, 50 grams olive oil (50 grams fat) failed to cause as great a cardiac acceleration as that which occurred after either of two salmon meals, *B* and *C*, each containing 21 grams of protein and 12 grams of fat.

of fat failed to elevate the heart rate to the level reached after each of two salmon meals containing 21 grams of protein and 12 grams of fat.

It seems logical to conclude for the present that fat foods vary in their cardio-accelerator action from animal to animal, and in that respect as well as in that of degree of action they differ from protein with its invariably intense and enduring accelerator action.

Variations in temperature in relation to these effects. It is well known that an increase in the temperature of the perfusate causes an increase in rate of the surviving, isolated heart. Knowlton and Starling (1912) found that in the heart-lung preparation of the cat a rise in the temperature of the perfusate from 36°C. to 41°C. was accompanied by a change in the

rate of the heart from 159 to 195 beats per minute, i.e., an acceleration of 36 beats or of approximately 7 beats per minute per degree of temperature change. Obviously it is highly improbable that the acceleration of the denervated heart after the ingestion of various foodstuffs could be explained solely on a basis of changes in the body temperature coincident with the oxidation of these foods. On that basis, a protein meal, which regularly causes an acceleration of 25 to 30 beats per minute, would have to cause a rise of more than 4°C. in the body temperature. We know that the normal animal so successfully maintains its heat regulation as to carry through the period of elevated metabolism after protein feeding without any marked change in its body temperature (Williams, Riche and Lusk, 1912). The animals used in this study were sympathectomized cats which differ considerably from normal animals in the efficiency of their heat regulating mechanism (Cannon, Newton, Bright, Menkin and Moore, 1929). This variation from normal, however, seems to be entirely in the direction of increased dissipation of heat rather than increased conservation. A sympathectomized cat has lost all power of reflex hair erection and of reflex peripheral vasoconstriction, two of the most important heat conserving mechanisms possessed by the normal cat, whereas the calorigenic action of the adrenals is also absent. The sympathectomized animal, therefore, finds difficulty in keeping up a normal body temperature, not in preventing the development of a supernormal one. During the colder months the sympathectomized cats, when brought to a warm room in the morning after a night spent in a rather chilly room, are regularly found to be shivering, and it is not at all unusual for them to exhibit under such conditions a subnormal rectal temperature, in some instances even as low as 36.5°C. As the morning progresses the animal manages slowly to restore its normal temperature of 39 to 39.5°C. This temperature is maintained until the animal is again exposed to cold. These facts were kept in mind during the performance of all the experiments reported in the previous parts of this paper. The feeding was never given until after the animal had spent several hours in a warm room, and throughout the experiment care was exercised to prevent the room from being cooled. Moreover, with several of the tamer animals, the rectal temperature was followed throughout the course of some of the experiments.

Figures 11 and 12 show curves of protein acceleration of the denervated heart together with the rectal temperature curve throughout the same period. In figure 11 the meal of liver was followed by a cardio-acceleration of 38 beats per minute, whereas the temperature varied only from 38.3°C. to 39.0°C. throughout the entire experiment, a temperature change which would account for an increment of only 5 beats per minute in the heart rate (Knowlton and Starling, 1912). Figure 12 shows an equally convincing experiment with cat 411. A sudden cold wave during the pre-

ceding night had chilled the animal rooms and when the cat was brought into the laboratory at 10:30 a.m. he was shivering and his rectal temperature was only 36.7°C. His heart rate was then 81 beats per minute as compared with his usual fasting heart rate of 90 to 95 per minute. He was placed in a cage upon which an electric heater was directed, and by 2:30 p.m. his rectal temperature had risen to 39.2°C. and his heart rate had increased to over 90 per minute. Then he was allowed to eat 100 grams of canned salmon. During the next two hours his heart rate increased from 91 to 113 beats per minute whereas during the same period his rectal tem-

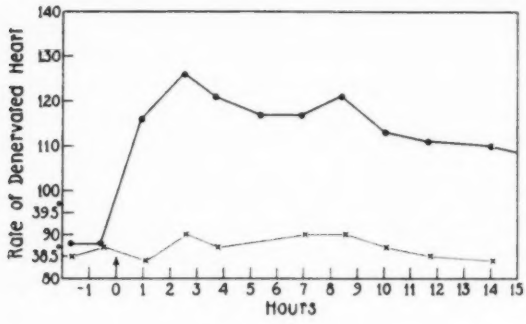

Fig. 11

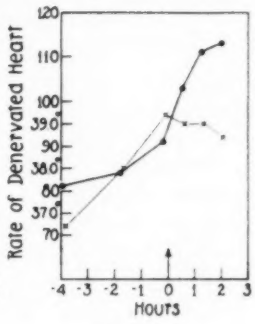

Fig. 12

Fig. 11. The heavy line indicates the rate of the denervated heart of cat 384 after the ingestion of 150 grams of liver (at the point marked by the arrow). The light line is a record of the rectal temperature variations during the same period. The maximum temperature variation throughout the entire experiment could account for a variation in heart rate amounting to only 5 or 6 beats per minute.

Fig. 12. Cat 411, after exposure to cold, had a rectal temperature of 36.7 C. An electric heater was applied and in 4 hours the temperature rose to 39.2°C. A meal of salmon, eaten at the point marked by the arrow, caused cardiac acceleration of 22 beats per minute during the next 2 hours, although the rectal temperature fell 0.5°C. Heavy line—rate of the denervated heart; light line—rectal temperature.

perature fell 0.5°C. Such results as these indicate quite definitely that the protein cardio-acceleration is a true chemical phenomenon and occurs independent of any temperature effects.

On the other hand we have observed rather marked rises in body temperature during the later stages of fat digestion, and in several instances these temperature changes quite obviously exaggerated and prolonged the apparent response of the heart to the fat meal. Such an effect is shown in figure 13. These instances have led us to suspect that the previously mentioned variation from animal to animal in the response to fat feeding may be largely an outcome of variations in the behavior of the body temperature during fat absorption.

In résumé, one may say that the changes in the body temperature coincident with protein digestion are never of sufficient magnitude to alter significantly the curves of heart-rate change if the temperature increment is deducted from the total acceleration. Furthermore, in those cases in which carbohydrate food has caused a significant increase in heart rate, temperature change has again been found to be insignificant. The temperature changes following fat ingestion are not such as to explain the initial rise in heart rate which fat produces, but, during the later hours of fat digestion, are in some cases of such character as to exaggerate the acceleration and to prolong its duration.

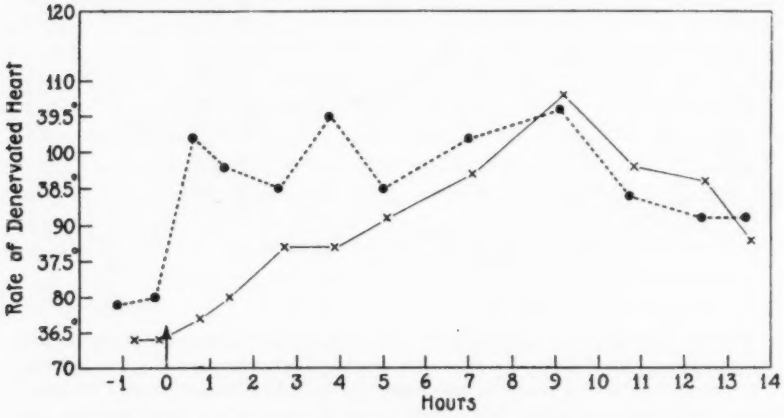

Fig. 13. Dotted line—rate of the denervated heart; continuous line—rectal temperature. A meal of butter was given at the time marked by the arrow. During the later hours of fat digestion there was considerable rise in body temperature which exaggerated and prolonged the apparent response to the fat meal.

Discussion. This paper has dealt with increases in the rate of the denervated heart following the ingestion of food. The question arises as to what these changes in rate may signify. The denervated heart can properly be considered as an isolated organ, differing from the heart of the Starling preparation in that it is isolated in the living body. As expressed by Cannon, Lewis and Britton (1926), "it is a continually and rhythmically active muscle, living isolated in the body fluids. Its rate is fixed by influences affecting a small portion of its structure, the sinus node. The changes in rate, however, are capable of revealing alterations in the bathing fluids, (the 'milieu interne,' to use Bernard's phrase) and, as accumulating evidence tends to prove, revealing such alterations as influence contractile structures in general and also in specific ways. In short, it

acts like an isolated organ perfused with different fluids—only it undergoes an *internal perfusion*."

Patterson, Piper and Starling (1914) found that the rate of the heart of the heart-lung preparation is, within wide limits, independent of the venous and aortic pressures, and "is determined entirely by the local condition of the heart muscle and is a function simply of the temperature of the pace maker." The rate of an isolated heart can be influenced, therefore, only through thermal or chemical changes in the perfusate. In a previous section it has been shown that the cardio-accelerations reported in this paper are in no way manifestations of temperature changes. Hence the inquiry into their nature resolves itself into a consideration of the chemical changes which occur in the blood following the ingestion of food.

Although it is possible that these accelerations may be the function of some component of one or other of the digestive juices, which, reabsorbed into the blood stream, acts specifically as a cardiac stimulant, it is hardly probable. As far as the writer knows, there is no evidence to show that any of the enzymes are cardio-accelerator agents. Furthermore, the extreme contrast between the effects of protein and those of carbohydrate food would be difficult to explain on this basis. Although differently composed foods evoke the secretion of digestive juices varying somewhat in their nature, these differences must be largely quantitative rather than qualitative, for the same secretin which brings about the secretion of pancreatic amylase also stimulates the secretion of the proteolytic and lipolytic enzymes of the pancreas (Bayliss, 1927). Again, there is the possibility that the transient alkalosis which occurs during gastric secretion might operate to produce this acceleration, especially in view of the fact that a decrease in carbon dioxide is associated with a faster rate of the isolated heart (Jerusalem and Starling, 1910). However, the protein accelerations are from 15 to 20 hours' duration whereas the secretion of hydrochloric acid in any quantity is limited to the early hours following meat ingestion. Dodds (1921) found that in the human subject the alkalosis which accompanies this acid secretion is of only 1 to 2 hours' duration, and, although figures are not available, it is very unlikely that in the cat it should persist for 15 to 20 hours. It seems that in the presence of more probable explanations one can reasonably set aside further consideration of the possibility of digestive juices or of post-prandial alkalosis operating to produce cardio-acceleration.

Certainly the most striking chemical changes in the blood incident to protein digestion arise from the absorption into it of the products of protein hydrolysis. Amino-acids (Van Slyke and Meyer, 1912; Folin and Berglund, 1922) and probably polypeptids also (Folin and Berglund, 1922; Kotschneff, 1926; Johnston and Lewis, 1928) enter the blood with sufficient rapidity to cause appreciable rises in its non-protein nitrogen

content. Van Slyke and Meyer (1913) have shown that the amino-acids are taken up by the tissue cells with great avidity. Their intermediary metabolism is known only in part, but it has been shown that the amino-groups which characterize them are in large part broken off and excreted as urea. The pharmacology of the amino-acids and peptids has been the subject of considerable investigation during the past twenty years. A review of the work already done in that field brings to light several interesting points in relation to the present problem.

It has long been known that when one or other of the artificial perfusing fluids is used, the isolated heart gradually becomes weaker in its action and enters into a "hypodynamic" state. Such a heart is immediately restored to activity if serum is substituted for the artificial perfusate. In 1878 Stiénon suggested that this restoration might in part be due to some albuminous substance in the serum. Popielski (1909) reported that the addition of Witte's peptone to a concentration of 0.0175 per cent caused a great increase in the rate and force of the beat of the perfused cat-heart. This effect of peptone was later confirmed in the rabbit, dog and frog (Yoshimura, 1912; Kondo, 1919). Popielski found, however, that the peptone contained 0.262 per cent of calcium in peptonate form, and that this amount of calcium if given in slightly dissociable form produced an effect identical to that of peptone. In applying Popielski's findings to the present problem one finds that "Cream of Wheat" contains 3 to 5 times as much calcium as does lean beef, and whole milk contains 15 times as much (Lusk, 1928). Moreover, the calcium of milk has been found to be completely ionized in the stomach and readily utilized by the tissues (Wha, 1924; Sherman and Hawley, 1922). Obviously, then, the effects of foods upon the denervated heart rate are not a function of their calcium content.

Lusanna (1910, 1911) found that high concentrations of glucose, urea, and all amino-acids would cause slowing and loss of irritability in the hypodynamic turtle-heart, whereas in lower concentrations glucose and urea were without effect while glycine, alanine, aspartic acid, leucine, tyrosine and phenylalanine restored strong contraction. In none of Lusanna's experiments was the hydrogen-ion concentration accurately controlled. Clark (1913), however, confirmed Lusanna's findings as regards glycine, using the hypodynamic frog-heart in experiments which were carefully controlled from the standpoint of acidity. Although at a pH of 8.3 glycine is an efficient "buffer," Clark found its restorative action much more powerful than that of other buffers, suggesting a rather specific action. Zunz (1910) reported that the proteoses nearest the protein molecule are not toxic for the turtle's heart, and that protalbumoses and synalbumoses have a restorative power. From these various reports it would appear that at least some of the products of protein cleavage possess stimu-

lant properties when applied to the cold-blooded heart. Their action upon mammalian heart preparations is still largely undetermined. The apparent effects of amino-acids upon the denervated heart of the cat will be considered in another connection. At this point it seems pertinent to consider another biological response to protein which is of great interest in regard to the effects described in this paper.

In 1780 Lavoisier demonstrated that there was an increase in oxidation in the body following the ingestion of food. Rubner (1885) showed that protein possesses the property of increasing body oxidations to a much greater degree than does carbohydrate or fat. He applied the term *specific dynamic* action to this property of a foodstuff to increase metabolism. Lusk, also, has emphasized the superiority of protein over carbohydrate and fat in this respect. It is desirable, however, to bear in mind that the metabolic changes following the administration of fat and carbohydrate, although inferior to those following protein, may nevertheless, under ideal conditions, assume considerable proportions. Lusk has found that glycine and alanine, administered orally, exert a specific dynamic influence. Leucine seems to possess the power to a slighter degree, whereas Rapport and Beard (1927) reported that tyrosine and phenylalanine stimulate metabolism, the latter to an even greater degree than does glycine. Rapport and Katz (1927) observed an increase in the oxygen consumption of the isolated, perfused leg of the dog following glycine administration, and concluded that its specific dynamic action "is a direct effect upon the cells of the tissues stimulated." On the other hand, Mann and Boothby (1928) found an absence of specific dynamic action in dehepatized dogs, whereas Guttmacher and Weiss (1927) believed the nervous system plays an essential rôle in the process. Lusk now (1928) concludes that protein food exerts an "*amino-acid stimulation*, in which some metabolites derived from protein stimulate the cells to a higher level of oxidative activity."

The question immediately arises as to whether the changes in the rate of the denervated heart which occur after the ingestion of food may not be manifestations of specific dynamic action. It seems reasonable to believe that they are. In the intact organism there is a close relationship in health and disease between the rate of the heart and the rate of the total metabolism (Benedict, 1915; Benedict, Miles, Roth and Smith, 1919; Harris and Benedict, 1919; Smith, 1922; Sturgis and Tomkins, 1920; Minot and Means, 1924). In the heart-lung preparation subjected to variations of temperature—between 32°C. and 39°C.—Evans (1912) found that "the rate of gaseous metabolism varies almost exactly as the pulse rate, the oxygen consumption and carbon dioxide production *per beat* being the same at both temperatures." Furthermore, the metabolism of the pace-setting region must be greater during a period in which it initiates 120 beats per minute than during a period in which it initiates 90. If protein products

increase the metabolism of cross-striated skeletal muscle(and since skeletal muscle constitutes over 40 per cent of the body weight (Skelton, 1927) its increase in metabolism must constitute a large factor in the total rise after protein feeding) might they not be expected to increase the metabolism of cross-striated cardiac muscle? And, in the absence of nervous influence, would not this increased metabolism of the sinus node, which consists of specialized cross-striated fibers (Keith and Flack, 1907), be reflected through an increased frequency of the heart-beat?

The effects of protein, fat and carbohydrate upon the rate of the denervated heart correspond closely to the well-known effects of these respective substances upon metabolism. In a large number of trials the cardio-acceleration following the ingestion of the several types of food has been found in every case to be of an extent which one might expect were this acceleration a direct function of the specific dynamic action of the respective food. Among many observations in numerous animals there has been no case in which the effects were inconsistent with this explanation. In three cases of protein feeding the gaseous metabolism was followed for six hours and the changes in heart rate were found to run parallel with it. It seems reasonable, therefore, to assume that the cardiac phenomena which have been observed are manifestations in this specialized tissue of the widespread changes which foods, and preëminently protein foods, have long been known to exert upon metabolism.

In the hope of securing further evidence in this direction a study of the effects of amino-acids upon the rate of the denervated heart has been made.[2] Unfortunately the work has been greatly complicated because the animals used as subjects have regularly failed to tolerate amino-acids administered by mouth. Even when given in dilute solution, glycine or alanine, in quantities comparable to the amounts required to raise metabolism have exerted a marked cathartic action. In the animals used, as described before, the splanchnic nerves and both abdominal sympathetic chains have been removed without interference with the left vagal distribution to the gut. Such animals are theoretically "vagotonic" as regards the intestinal tract. Although under normal diet no tendency to diarrhea is observed, there may possibly exist some degree of hypersensitivity to agents which normally excite peristalsis. Be that as it may, it has been consistently found that 1 or 2 grams of glycine or alanine, whether in aqueous solution or mixed with some carbohydrate food, exerts a marked purgative action and is being passed *per rectum* within 20 or 30 minutes. Although on different occasions a slight acceleration of the denervated heart was observed immediately following the oral administration of these substances, coinci-

[2] The amino-acids used were purchased from the Eastman Kodak Company, Rochester, New York.

dent with the expulsion of large quantities of fluid from the rectum this acceleration disappeared and the results of the experiments, therefore, were not decisive.

This difficulty has necessitated the administration of the amino-acids through parenteral routes. When glycine is injected intravenously at rates comparable to that at which its absorption into the blood during protein digestion might occur, i.e., in physiological doses, it appears to produce no change in the rate of the denervated heart. For example, using the Colwell apparatus for constant injection, 10 per cent glycine was given intravenously (without anesthesia) to cat 403 at the rate of 10 cc. (1 gram of glycine) per hour. The heart rate remained at its fasting level throughout the period. During two one-hour periods later in the day injections at the rate of 30 cc. (3 grams of glycine) per hour again failed to produce acceleration. Quite obviously, then, the prolonged cardio-accelerations which this animal showed following meals of protein (see figs. 3a and 7) were not a pure and simple function of the glycine content of the blood. But on the other hand the reports of specific dynamic effects upon total metabolism after the intravenous administration of glycine are far from convincing.

It has long been known that many substances, when injected intravenously, may cause very material increases in heat production. Scheremetjewski (quoted by v. Mering and Zuntz, 1877) observed such effects from glycerine, sodium lactate and sodium caproate. Tangl (1911) reported similar action of 5 per cent sodium chloride which brought about 15 to 35 per cent increases in heat production, and 5 per cent urea which produced increases of as much as 27 per cent. Verzár (1911) confirmed Tangl, noting considerable increases in heat production following the intravenous administration of 0.75 per cent sodium chloride and still greater increases when the salt was administered in higher concentrations. Krzywanek (1923) found that 0.75 per cent solutions of sodium chloride, sodium sulphate or sodium dihydrogen phosphate produced increases of 10 to 15 per cent in the gaseous exchange over at least a 2 to 3 hour period and that distilled water also brought about measurable effects. There was no considerable blowing off of carbon dioxide in these experiments. They seem to show conclusively that when one introduces a substance intravenously to determine its effect upon metabolism, careful attention must be paid to the disturbances it may cause in the osmotic and acid-base equilibria of the blood before the effects which follow its administration may be definitely interpreted.

Of seven groups of investigators who have reported studies on the effects of intravenously injected amino-acids upon metabolism, six obtained results which they interpret as pointing to a specific calorigenic influence. Liebeschütz-Plaut and Schadow (1926) found that the administration of 0.2 to

7.2 grams of glycine or alanine into the femoral vein (local anesthesia) of dogs produced no change in total metabolism, although dynamic effects were observed after the administration of like amounts via a duodenal fistula. Wolf and Hele (1914) gave 5 grams of glycine (in 120 cc. physiological saline) to dogs decerebrated by the Langley starch method but an analysis of their results does not verify their report of a specific dynamic effect. Krzywanek (1923), Weiss and Rapport (1924), Wilhelmj and Bollman (1928) and Mulert (1929) report rises in metabolism after the intravenous administration of amino-acids to normal animals, and Aub, Everett and Fine (1926) report similar results in decerebrate cats. All of these observers used glycine or alanine in 5 to 10 gram doses, injected as a solution of from 8 to 20 per cent concentration. In the great majority of cases the changes observed were fleeting, much shorter in duration than the effects regularly given by proportionate quantities of protein. None of the experiments were controlled in relation to the effects of high salt concentrations or of acid solutions. The amino-acids are salts and furthermore they are acid substances. Although they are but weakly dissociated acids, their acidity cannot be disregarded. Clark (1913) has shown that the "buffer" action which glycine exerts in solutions having a pH of 8.3 is insignificant at the hydrogen-ion concentration of the blood stream. Weiss and Rapport (1924) found that following the intravenous administration of 10 grams of glycine to a dog, the respiratory quotient rose during the second half-hour to 1.25 in one experiment and to 1.15 in another. There was, then, a blowing off of carbon dioxide, indicating, as the authors stated, "a neutralization of the alkaline carbonate by the glycine." Evidently, therefore, glycine behaves in the blood stream as an acid. Moreover, these experiments show that the disturbance in the acid-base equilibrium of the blood after the injection of 10 grams of glycine, since marked changes were present in the second half-hour, is not necessarily compensated rapidly, and that it is associated, at least temporally, with the increased oxidation which the glycine causes. Aub, Everett and Fine (1926) reported rises in the total metabolism of the decerebrate cat lasting for only 0.5 to 1.5 hours after the intravenous injection of 5 grams of glycine dissolved in 50 cc. of mammalian Ringer solution. They found that 5 grams of the dicarboxylic glutamic acid, *the solution of which was carefully brought up to a pH of 7.4 with sodium carbonate*, produced no effect, and concluded that glycine exerted a *specific* dynamic action whereas the glutamic acid did not. It seems that this conclusion would have been less equivocal if both solutions had had the same molecular strength and the same hydrogen-ion concentration.

Rather marked changes in the rate of the denervated heart of the cat have been observed following rapid intravenous injections of concentrated solutions of glycine. Five cubic centimeters of 20 per cent glycine (1 gram

of glycine) produced repeatedly in cats 403 and 384 an immediate 10 to 20 beat acceleration in heart rate with a return to normal in 30 to 40 minutes. But this effect could be duplicated by the injection of a similar volume of an equimolecular glucose solution (5 cc. of 48 per cent or 2.4 grams). Evidently the effect was not specific. If the same amount of glycine was injected slowly over one hour no change in heart rate occurred, and the same absence of effect accompanied a similar slow injection of the glucose. When the amount of glucose was trebled, i.e., when 7.2 grams were injected during one hour, there occurred a cardio-acceleration of 10 beats per minute during the latter part of the period. The blood sugar at the time of this acceleration was 476 mgm. per cent. The glucose, obviously, caused an acceleration of the heart only when it was present in the blood in a concentration far above the physiological limit. Likewise, the authors cited above have determined the effect of amino-acids upon metabolism in concentrations far above the physiological level, i.e., the blood amino-acid level following the ingestion of meat.

Until the effects of intravenously administered amino-acids upon metabolism are shown to be definitely independent of their saline properties and of their possible effects upon the hydrogen-ion equilibrium of the blood, and until effects comparable to those produced by protein food are shown to occur when the acids are administered at a rate approximating that of their absorption from the intestine during protein digestion, it would seem premature to maintain that the duplication of a protein effect by the intravenous administration of amino-acids is prerequisite to regarding that effect as a manifestation of specific dynamic action. Unfortunately, in the animals used in the present study, the purgative action of amino-acids has prevented administering them by mouth. A survey of the heart-rate curves obtained after the ingestion of meat, however, seems to lead to the rational assumption that these protein effects upon the heart are part of the well-known action of protein as a metabolic stimulant.

Although a faster pulse was observed after rapid injections of glycine, its intravenous administration at rates approximating the probable rate of amino-acid absorption from the intestine during protein digestion failed to produce any change. Yet a protein meal caused large accelerations in the same animals. If glycine may be considered as a representative amino-acid, then, in view of the close association which exists between the rate of the sinus region and its metabolism, it is hard to believe that the specific dynamic action of protein upon the tissue cells is a simple function of the presence or the metabolism of amino-acids.

Of course, glycine is only one of the amino-acids. Lusk (1912–13) found, however, that given orally it increases the metabolism of the dog, and since that time glycine has been widely used by students of metabolism as a typical amino-acid. Furthermore, the cardio-acceleration caused by

protein may be the result of a specific pharmacological property of some other amino-acid, a property not possessed by the group as a whole. The close relationship between the chemical structure of tyrosine or of phenylalanine and that of adrenin might cause one to suspect these acids in this connection, but the careful studies of Lusanna (1910–11) failed to reveal any difference between their action and that of glycine when applied to the cold-blooded heart (p. 532). Experiments are now being performed with various amino-acids to see if any of them possess a specific cardio-accelerator power when given intravenously to cats with denervated hearts.

As described before, moderate degrees of cardio-acceleration occurred after the feeding of carbohydrate and of fat. The interpretation in terms of specific dynamic action can be reasonably extended to include these results also. Carbohydrate and fat are both known to possess the property of increasing metabolism when fed in proper quantities. Their effects upon metabolism occur less constantly than do those of protein and are of much less magnitude. Although Abelin (1924) assigns to fat a place below carbohydrate in the scale of specific dynamic power, one gathers the opposite impression from Lusk's recent review of the subject (1928). The changes observed in the denervated heart would tend to support the latter view, i.e., that fat occupies a position somewhere between that of carbohydrate and that of protein in its power to increase body oxidations. The fat effects, however, have been very inconstant and have varied from animal to animal in a striking fashion. Furthermore, as noted before, the body temperature often underwent a rise during the later stages of fat digestion and this fact further complicates the interpretation of the results. It can be safely said, of course, that neither of the non-nitrogenous foodstuffs approaches protein in its power to accelerate the heart Further observations will be necessary, however, before one can say which of the two, carbohydrate or fat, exerts the lesser cardio-accelerator action.

SUMMARY

The cardio-accelerator effects of foods were determined in cats in which the heart was so completely denervated as to exhibit no significant change in rate when the animal passed from a state of bodily rest to one of very active muscular exertion combined with extreme emotional excitement.

Carbohydrate foods (see figs. 4, 5, 6, 7 and 8) and fats (see figs. 8, 9 and 10) were found to cause, in some instances, an acceleration of the denervated heart of the surviving unanesthetized cat. These changes, however, do not approach either in magnitude or in duration the effects which have been found to follow the administration of protein (see figs. 2, 3a, 3b, 4, 5, 6, 7, 8, 9, 10 and 11).

Protein foods invariably bring about an acceleration of surprising mag-

nitude and duration. After a meal of meat the increase in heart rate regularly amounts to a 25 to 50 per cent rise above the fasting level and persists for 15 to 20 hours to reach a total of 13,000 to 22,000 extra heart beats (figs. 3a and 3b). In other words, a protein meal throws an extra burden of work upon the heart, which, providing other factors than rate remain constant, is comparable in extent to the heart's total performance during 3 or 4 hours under fasting conditions. Obviously, a high protein diet is incompatible with cardiac rest.

The cardio-accelerations which follow the administration of protein or of carbohydrate are not conditioned by any rise of body temperature accompanying the oxidation of these foodstuffs (see figs. 11 and 12). Although changes in body temperature do not account for the initial rise in heart rate following the administration of fat, a rise in temperature which may occur during the later hours of fat digestion often serves to exaggerate the apparent effect of the fat-feeding and to prolong its duration (see fig. 13).

In view of the close association which must exist between the rate of the pace-setting region and its metabolism it has seemed reasonable to interpret these accelerations in terms of specific dynamic action. Thus whereas fat and carbohydrate may increase the metabolism of heart muscle to a measurable degree, it is protein which here, as elsewhere, excels as a stimulus to oxidation.

Glycine, when administered intravenously at very rapid rates, causes an acceleration of the denervated heart (see p. 536). Rapid injections of equimolecular solutions of glucose produce the same effect. When glycine is injected at a physiological rate, i.e., at a rate approximating that of amino-acid absorption into the blood stream during protein digestion, no change in heart rate occurs, and, as a corollary, no change in the metabolism of the sinus node (see p. 535). A review of the literature upon the increases in total metabolism following the intravenous administration of glycine reveals that glycine has commonly been administered in rates greatly exceeding the physiological (see pp. 535 to 537). It is suggested that the changes in body metabolism following such injections may be a function of disturbances in the acid-base and osmotic equilibria of the blood, and do not necessarily point to any specific stimulating power of the glycine.

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STUDIES ON THE ANEMIA OF RICE DISEASE IN RATS

The Influence of Vitamins A, B, D, Iron, Copper, Beef Muscle, and Liver on the Course and Regeneration from the Anemia of Rice Disease

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Recent developments in the treatment of anemias of the "idiopathic" variety (Minot and Murphy, 1926), and the realization of the hematopoietic importance of dietary deficiencies, have resulted in a renewed interest in the experimental study of anemia of both the primary and secondary types. Experimentally, a number of procedures have been found to reduce the erythrocyte count and the blood hemoglobin. The degree of anemia thus produced, although varying rather widely, in general, is proportional to the severity of the causative method used. Such procedures include direct reduction of the blood elements by repeated hemorrhage (Whipple and Robscheit-Robbins, 1927), reduced food intake (Morgulis, 1923), deficient iron intake (Hart et al., 1927), and various vitamin deficiencies. Each method has a more or less limited clinical application, i.e., anemia by hemorrhage requires the repeated removal of large volumes of blood in addition to a restricted diet; the milk diet utilized by Hart and co-workers is applicable only to young animals at a definite stage of growth, while the reduction of the caloric intake is usually complicated by a certain degree of vitamin deficiency and both in turn with inanition. Nevertheless, specific vitamin deficiencies in an otherwise balanced diet offer an accurate procedure for the study of anemia. Koessler et al. (1926) were able to induce an anemia in rats by restricting the vitamin A content of the diets fed. The addition of the deficient factor resulted in a remission. Damianovich, Bianchi and Savazzin (1923) also noted an anemia from avitaminosis in rats and, although the degree of erythrocyte reduction was slight with vitamin A deficiency, a much stronger anemia developed in the absence of vitamin B. The addition of the latter factor was also followed by a more marked recovery. These results have been confirmed for pigeons by Barlow (1927) who observed that an exclusive

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polished rice diet (primarily deficient in vitamin B) resulted in a 25 to 75 per cent reduction of the red cell count and a comparable reduction of the hemoglobin level. These results seemed to merit a study of the blood picture of mammals receiving a similar diet, both from the standpoint of a specific vitamin deficiency and from that of beri-beri.

In the present study, albino rats were subjected for a prolonged period to a polished rice diet to which (control series excepted) either cathartics or various dietary correctives were added. After the degree of inanition had progressed to near the margin of safety (60 to 70 per cent of the normal weight level) the influence of various therapeutic measures on the rate of blood regeneration from the anemia invariably observed in untreated animals was noted.

METHODS. Adult male rats ranging from 175 to 300 grams in weight were placed on a mixed diet of the Osborn-Mendel type (Sollmann, Schettler and Wetzel, 1920) for a sufficient period to establish a basal weight level. The control observations, taken bi-weekly during this period, included body weights, erythrocyte counts and grams of hemoglobin (Newcomer method) per 100 ccm. of blood. The animals were then segregated in groups of 3 to 5 animals each and the diet so altered that the influence of vitamins A, B, A and B, and the metallic elements Cu and Fe on the development of rice anemia could be noted during the succeeding 10 week feeding period. The diets were as follows: *group 1*, dry (uncooked) rice; *group 2*, cooked rice and salts (1 per cent NaCl and 2 per cent $CaCO_3$); *group 3*, cooked rice, salts and 1 gram of cod liver oil per rat daily; *group 4*, diet same as that of *group 3*, with 2 per cent yeast (Fleishmann's compressed); *group 5*, cooked rice, salts and 2 per cent yeast; *group 6*, cooked rice, salts and ferrous ammonium citrate (equivalent of 10 mgm. of Fe per rat daily); *group 7*, cooked rice, salts and $CuSO_4$ (0.01 mgm. per rat daily), and *group 8*, cooked rice, salts, Cu and Fe in the dosages given for group 7 and 6, respectively. During this period the weights were taken weekly while the red cell counts and hemoglobin levels were determined at two week intervals. On concluding this 10 week study, the influence of various therapeutic measures, including dietary additions of liver, beef muscle and yeast on the rate of blood regeneration of the same series of animals was observed.

RESULTS. *The influence of polished rice on the body weight, erythrocyte counts and hemoglobin levels* with time are shown in figure 1. The weight loss of animals fed uncooked (*A*) or cooked (*B*) rice did not differ noticeably during the ten weeks of the feeding period *f.i.*, the median weight losses were 34 and 31 per cent, respectively. These slight differences are probably within the range of experimental variation. The changes noted in the erythrocyte counts of the two groups, although showing a 5 per cent variation at the 6 week period, were of the same order of magnitude at the

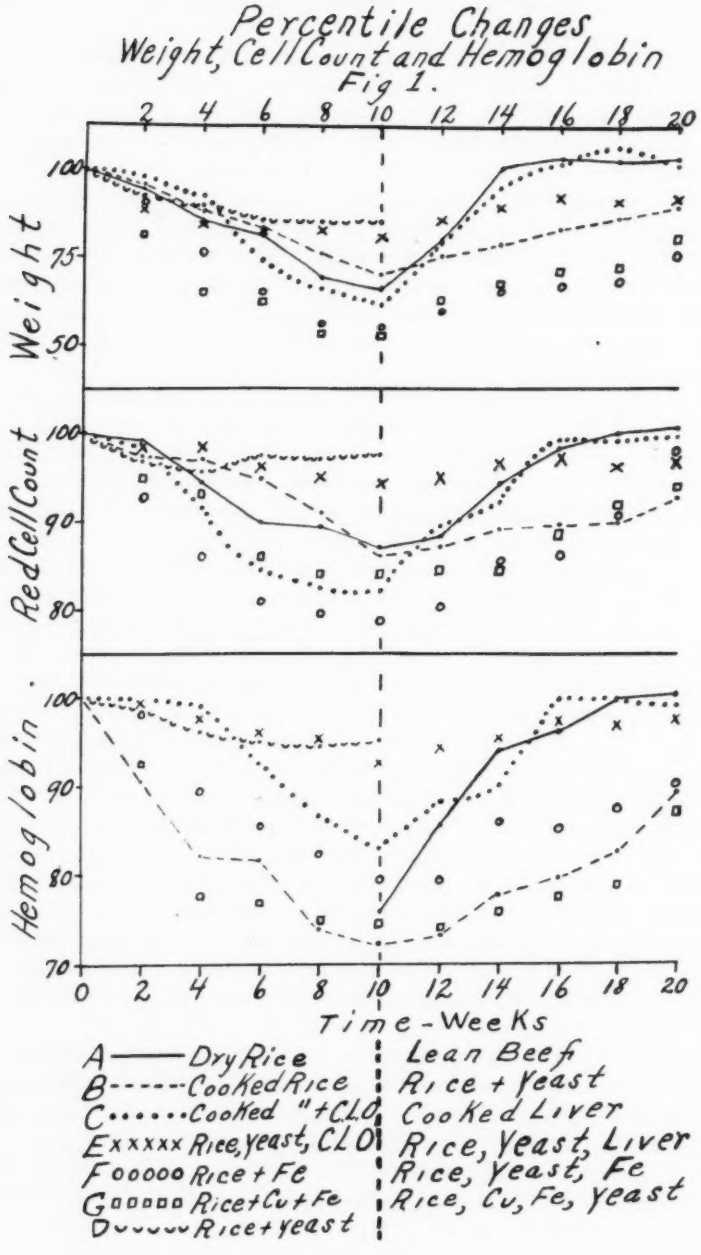
Percentile Changes
Weight, Cell Count and Hemoglobin
Fig 1.
Weight
Red Cell Count
Hemoglobin
Time - Weeks
A —— Dry Rice
B ----- Cooked Rice
C ..... Cooked " + CLO
E xxxxx Rice, yeast, CLO
F ooooo Rice + Fe
G □□□□□ Rice + Cu + Fe
D ~~~~~ Rice + yeast
Lean Beef
Rice + Yeast
Cooked Liver
Rice, Yeast, Liver
Rice, Yeast, Fe
Rice, Cu, Fe, Yeast

Fig. 1

10th week, i.e., a median decrease of approximately 15 per cent from the normal was observed with each group. The decrease of the hemoglobin level from a normal level of 15.6 to 11.5 grams per 100 ccm. of blood although proportionately greater than that noted for the red cell counts did not differ significantly for the two groups.

The addition of cod liver oil in a daily dosage of 1 ccm. per rat, as a source of vitamin A (*C*), to a cooked rice diet resulted in an apparent initial delay at the 4 week period in the weight loss usually observed in the absence of this factor and subsequently a more rapid development of inanition. At the 10th week the weight loss of the control series was 32.5 per cent while the rice and *CLO* (cod liver oil) series showed a 38 per cent loss. The changes in the red cell counts of the rice and *CLO* group (*C*) were greater than those of the control series (*B*) and correspond roughly to the weight changes noted. The decrease in the hemoglobin level of the *CLO*-rice group was of the same order as that of the cell counts of the same group. The addition of cod liver oil to the rice diet was obviously of no benefit and actually accentuated the development of inanition due to a partial refusal of the animals to eat the diet.

The correction of the vitamin deficiency of the rice diet by adding compressed yeast, in a proportion of 2 per cent of the daily food intake (fig. 1-*D*), practically arrested the course of inanition observed in the absence of this factor (weight loss at the 10th week between 5 and 10 per cent) and in addition limited the degree of anemia which developed. The cell counts of the controls, series A and B, showed a decrease of 14 per cent while the rice and yeast group showed a loss of only 3½ per cent at the 10th week. The maintenance of the hemoglobin level by the addition of yeast to the diet paralleled that of the erythrocyte counts.

The correction of both the vitamin A and B deficiencies of the rice diet is shown by figure 1 (*E*). The weight changes observed were midway between those of the rice and yeast series and the rice and *CLO* group, while a slightly greater anemia was noted in the rice, yeast and *CLO* fed animals than with rice and yeast only. The data apparently illustrate two points; first, the tendency of the B factor to limit the course of inanition and the development of anemia, and secondly, the acceleration of these reactions by the addition of cod liver oil. The results with cod liver oil, however, are somewhat misleading and are actually due to a refusal of the animals to eat a sufficient quantity of the diet containing the proportions of the oil fed.

The addition of iron to the rice diet. The therapeutic dose of iron as ferric ammonium citrate was added to the standard rice diet primarily because of a comparable iron content of the rice and the milk used by Hart et al. for a similar purpose and secondarily because of the evident beneficial action of iron administered by Whipple to dogs and by Mitchell and Schmidt (1926) to rats after the development of anemia by hemorrhage. The

results obtained are illustrated by figure 1 (*F*). The weight loss of the rice and Fe group was 13 per cent greater than was observed with rice alone. The erythrocyte counts of the rice and Fe group also showed a greater decrease (to 78 per cent) than those of the control series (to 86 per cent). The hemoglobin changes on the other hand were 5 per cent less with Fe group than with the control series. The significance of the latter observation because of the smallness of our series is not established.

The addition of Cu and Fe to the rice diet was made with the expectation that the two ions might prove more beneficial than iron alone. The results are illustrated in figure 1 (*G*). The greatest weight loss observed was noted with this series, i.e., greater than with the control series or with iron only. The erythrocyte changes with rice, Fe and Cu were less than with iron alone but greater than with the control groups (*A* or *B*). The hemoglobin changes paralleled those of the control series B and were greater than were observed with a rice and iron diet. It is therefore apparent that neither iron nor copper are of benefit in so far as hematopoietic or nutritional changes of rats on a beri-beri diet are concerned, i.e., a depletion of the body iron reserve was not evident during the 20 week period of this study in spite of the almost iron free character of the diet. We observed that the animals receiving the metals and rice actually ate less food than the controls, i.e., voluntarily fasted perhaps because of the taste of the diet. However, our results may be due simply to experimental variation.

The comparison of yeast, lean beef muscle, and liver on the nutritional recovery and hematopoietic regeneration was made by substituting lean beef for rice (*A*), addition of 2 per cent yeast to rice (*B*), substitution of liver for rice and *CLO* (*C*), substitution of liver for *CLO* in diet of group *E* and addition of yeast to diets of groups *F* and *G*. The order of nutritional efficiency of these procedures from greatest to least was lean beef muscle, liver, yeast, liver added to rice and yeast, yeast added to rice, Cu and Fe, and yeast added to rice and iron. The response to lean beef and to liver in *A* and *C* was most striking both in point of time and degree. The weight recovery was complete in 4 weeks. With the other procedures tested, recovery was more gradual and was incomplete after 10 weeks of study.

The therapeutic order of efficiency of the curative measures on the erythrocyte counts from greatest to least was lean beef, liver, yeast added to rice, Fe and Cu; yeast added to rice; and liver added to rice and yeast (*C*). The red cell regeneration on the improved dietary definitely lagged behind the weight changes, *f.i.*, with lean beef or liver complete recovery was observed two to three weeks after the body weight level had become normal. A similar delay was likewise observed with all measures tested and on terminating the study, the level of the red cell counts, although subnormal, were directly comparable to the degree of nutritional improve-

ment. The curve of hemoglobin regeneration corresponds closely to that observed for erythrocytes and the slight divergences noted are probably insignificant. The addition of yeast to the diets of *B*, *F* and *G* produced a comparable although incomplete recovery in each series, i.e., from a median value of 76 per cent for the three groups to 89 per cent of the normal values.

CONCLUSIONS

A secondary anemia was induced in rats by feeding a diet consisting of polished (cooked) rice and salts. With such a dietary, the body weight and hemoglobin values were diminished to a greater extent than the red cell counts.

The addition of 2 per cent compressed yeast to polished rice forms a diet which is practically balanced for rats. The erythrocyte counts and hemoglobin levels are maintained within a normal range and the body weight losses are much less than with rice alone. Yeast, however, is less active as a curative than as a prophylactic measure in rice disease.

Additions of vitamins A and D in the form of cod liver oil were ineffective in preventing the onset of polished rice anemia.

Therapeutic doses of iron or copper either singly or in combination are ineffective either as curative or prophylactic measures in rice anemia.

Lean beef muscle or liver either added to the rice diet or substituted completely for the cooked rice resulted in a more rapid and complete remission of the anemia of rice disease than was observed with any other procedure tested.

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A COMPARISON OF THE BODY WEIGHTS, ERYTHROCYTE COUNTS AND TOTAL BLOOD VOLUMES OF NORMAL, BERI-BERI AND FASTING RATS. THE INFLUENCE OF LACTOSE, MINERAL OIL, AND MAGNESIUM CARBONATE ON THE ANEMIA OF RICE DISEASE

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The administration of magnesium sulphate, lactose or mineral oil has been shown to prevent and remove the anemia of rice disease in a large percentage of the pigeons studied (Barlow, 1927). The beneficial action of these agents was tentatively attributed to catharsis with a possible limitation of bacteriemia. Subsequently it was shown that the cathartics did not produce a blood concentration at the time the observations were taken (Barlow and Biskind, 1928) and consequently they were true prophylactics in counteracting the anemia of rice disease. It appeared interesting to determine whether similar effects could be secured in mammals. Experiments analogous to those reported in the study with birds were performed on white rats.

METHODS. Male albino rats of various ages, ranging from 70 to 359 grams in weight were placed on a balanced diet (Sollmann, Schettler and Wetzel, 1916) for a sufficient period to establish basal weight levels for the adult animals and to verify the normal growth curves of the smaller rats. The control observations taken bi-weekly during this period included weight, erthyrocyte counts, hemoglobin in grams per 100 cc. of blood (Newcomer method) and whole blood specific gravity—Hammerschlag.

In the first study, the influence of cathartics on the development of rice disease was noted, to determine whether a beneficial action similar to that observed for pigeons could be demonstrated with rats. With this object in view, four groups of 4 rats each were placed on a cooked rice diet plus salts for a four week period. The diet of group 1 thereafter remained unchanged but to the diets of the other animals laxatives were added in the following daily dosages per rat: group 2, 1 ccm. of mineral oil; group 3, 1 gram of mag-

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nesium carbonate; and group 4, 1 gram of lactose. The possibility of the cathartics producing a blood concentration was eliminated by discontinuing their addition for 24 hours immediately preceding any observations taken on the blood. The experiments were continued as long as practicable.

In the second or blood volume study, the rats were divided into six groups according to the diet and treatment received, as follows:

GROUP	NUMBER OF ANIMALS	DIET	PERIOD OF STUDY
1	10	Mixed whole grain	3 to 4 weeks
2	9	Cooked (P) rice and salts	4 to 10 weeks
3	21	Fast with access to water	2 to 12 days
4	4	Rice and 1 cem. mineral oil daily	4 weeks
5	3	Rice and 1 gram $MgCO_3$	4 weeks
6	4	Rice and 1 gram lactose	4 weeks

At the termination of the observation period of each respective group, the animals were lightly anesthetized with ether, cannulae inserted and the total blood volumes determined by the modified Welcker method of Barlow and Biskind (1928).

RESULTS. *The influence of cathartics on the anemia of rice disease is illustrated by figure 1.* The four groups of rats placed on a diet of cooked rice and salts showed a variable weight loss at the end of the four week preliminary feeding period, i.e., from 9 to 27 per cent. This variation was obviously due to differences in the appetites of the respective animals for the rice. The administration of mineral oil, magnesium carbonate or lactose to groups 2, 3 and 4 did not alter, during the six weeks of their administration, either the direction or the degree of the inanition curves established preceding the dietary changes.

The degree of anemia as shown by the decline of the median erythrocyte count in each group was slight during the preliminary rice feeding period. The addition of magnesium carbonate, lactose or mineral oil in a daily dosage equivalent to 1 gram per rat per day to the cooked rice and salts diet obviously diminished the rate of decrease of both the erythrocyte and hemoglobin values, and apparently antidoted partially or delayed the development of the anemia usually observed in the absence of these cathartics. The prophylactic efficiency of the three cathartics as judged by the cell count and hemoglobin values has the following order from the greatest to the least: lactose, $MgCO_3$ and mineral oil.

A comparison of the weights, erythrocyte counts, total red cells and blood volumes of rats receiving normal, and berberi diets with and without the addition of cathartics is made in figure 2. Normal diet. The body weights of the control series of 10 adult animals ranged from 221 to 359 grams (med-

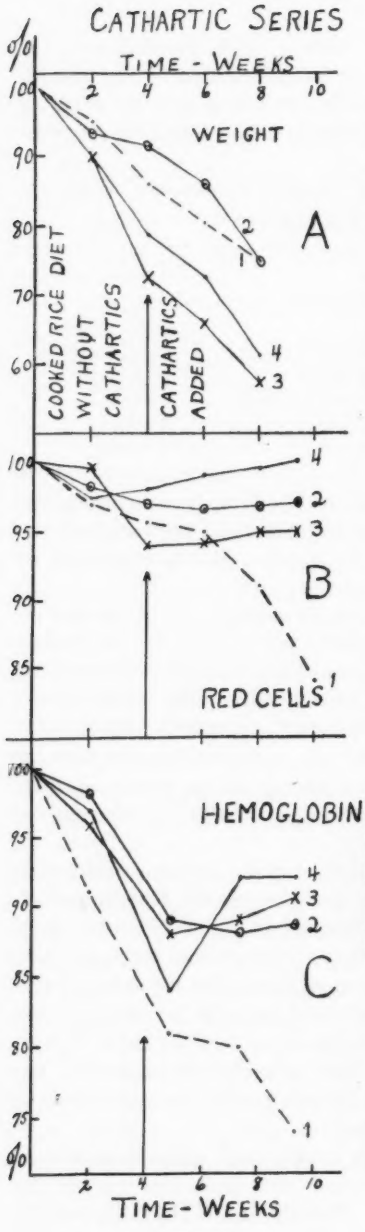
CATHARTIC SERIES
TIME - WEEKS
WEIGHT
COOKED RICE DIET WITHOUT CATHARTICS
CATHARTICS ADDED
A
B
RED CELLS
HEMOGLOBIN
C
TIME - WEEKS

Fig. 1

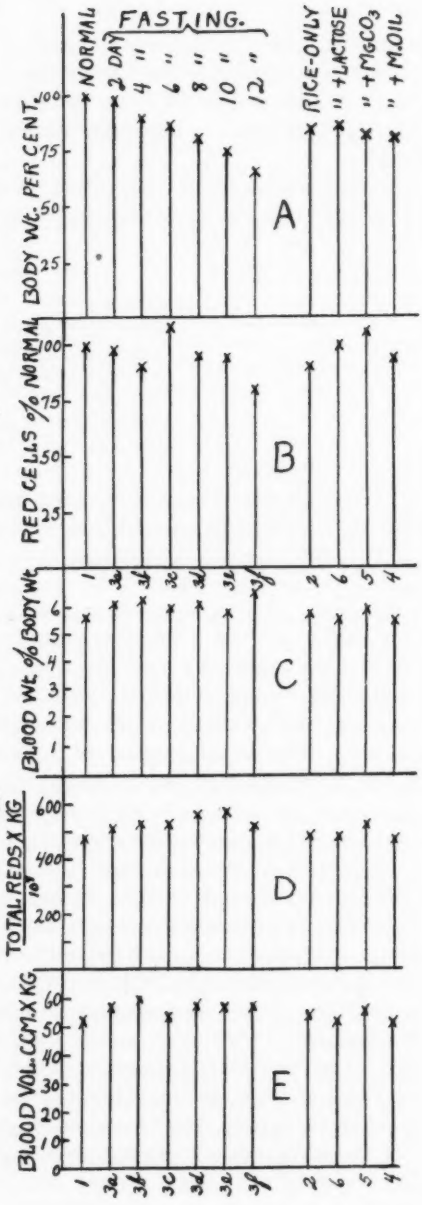
FASTING.
NORMAL
2 DAY
4 "
6 "
8 "
10 "
12 "
RICE-ONLY
" +LACTOSE
" +MgCO3
" +M.OIL
BODY WT. PER CENT.
RED CELLS % NORMAL
BLOOD WT. % BODY WT.
TOTAL REDS X KG
BLOOD VOL. CC/M X KG
A
B
C
D
E

Fig. 2

ian 282). The median red cell count for the group was 8,672,000. The observed blood volumes determined by the washout method, ranged from 50 to 60.6 (median 52.5) cubic centimeters per kilogram body weight. The total blood weight corresponded to 5.6 per cent or slightly more than 1/18th of the total body weight. The median blood volume figure obtained by the authors is 7.7 per cent lower than similar data obtained by Chisholm (1911) in a comparable manner from rats ranging from 200 to 300 grams in weight. The deviation of individual data from the mean was rather small in our experiments but it is possible that our lower figure for total blood volume is due to the smallness of the series.

The influence of a beriberi diet on the body weight and blood picture. The administration of a cooked polished rice diet to rats (fig. 2, *2*) over a four week period results in a 16 per cent loss of body weight, A, an 8 per cent decrease in the red cell count per cubic millimeter of blood and a slight but definite increase in the volume of blood in cubic centimeters per kilogram of body weight, *E*. The blood volume was maintained approximately at the normal ratio during the rice feeding period and the apparent increase in blood volume is partially explained by the disproportion existing between the blood and body weight changes as inanition progresses. This relationship becomes more striking as the severity of inanition increases, i.e., with a 12 to 16 week rice feeding period. The small decrease in the red cell count is due partially to a relative but not an absolute anemia and partially to the development of a mild hydremia, i.e., the cell count per unit volume of blood is diminished although the total or absolute cell count per 100 grams body weight corresponds very closely to similar data of normal animals, while the total blood volumes are slightly greater than normal. This condition becomes more accentuated with the severity of inanition (as with prolonged fasting or rice feeding) and under such conditions is accompanied by the development of both a relative and an absolute reduction of the erythrocyte count and hemoglobin level. The total blood weight of normal rats corresponds to approximately 5.6 per cent of the gross body weight. Corresponding data after a four week rice feeding period has a value of 5.74 per cent and after a seven to ten week feeding period the value rises to 6.21 per cent. Similar changes were noted by Scott and Barcroft (1924), with rats rendered anemic by means of a white bread and whole milk diet.

The addition of lactose to the polished rice diet (*A*, 6) did not influence the progress of inanition usually observed with rice alone (*A*, 2), while the cell counts were maintained at normal levels (*B*). The addition of lactose to the diet apparently prevented the development of the blood changes which usually occur in its absence, since the red cell count per cubic millimeter and the total blood volume was maintained within normal limits. Neither was any evidence of hydremia noted since the total red cell count and the relation between blood and body weights remained normal.

Addition of magnesium carbonate to the rice diet accentuated the progress of inanition (*A*, 5 and 4), but definitely delayed or prevented the development of rice anemia (*B*). The slightly increased red cell counts per unit volume of blood as well as the total red cells per kilogram body weight (*D*) are apparently not due to blood concentration since the total blood volume (*E*) is 6 per cent greater than that of our normal rats. Magnesium carbonate apparently tends to accentuate the hydremic tendency of rice disease but at the same time may limit the rate of destruction of red cells, since the total number of red cells per unit body weight was definitely increased.

Mineral oil added to a polished rice diet, by decreasing the appetite of the animals hastens the progress of inanition. The red cell count, however, was maintained near the normal level, i.e., slightly higher than with rice alone. The apparent prophylactic action of mineral oil during the period of the experiment was negligible since the total red count of this group was less than similar observations of either control series, i.e., normal or rice (1 and 2). The beneficial action of mineral oil on the anemia of rice disease illustrated in figure 1, was relatively insignificant and could not be demonstrated in the short (4 week) feeding period of the blood volume study.

The influence of various degrees of acute inanition on the body weights and blood pictures of normal rats is illustrated in figure 2 (3a to f). The median weight changes are roughly proportional to the length of the period of inanition. The degree of change, however, is closely associated with the age and size of the animals since young or small rats starve more quickly and in addition show a smaller maximal weight loss at death than adult or larger animals. During the first two days the weight loss is minimal, due to a slightly greater water intake, and approximates 2 per cent of the original weight per day. After the 2nd day the weight loss corresponded to approximately $2\frac{1}{2}$ per cent of the original weight values per day and during the last 24 to 48 hours the degree of loss is usually maximal since the weakness of the animals limits the water intake.

The erythrocyte changes (*B* and *C*) like the body weight are closely associated with the age and size of the animals. In general, however, if corresponding inanition periods of both large and small animals are compared only quantitative differences in the blood picture are observed. After the second day of fasting, the cell counts decrease progressively with time. The changes are negligible with short fasting periods but with prolonged fasting (8 or more days) the values reach 90 to 75 per cent (median 14) of the normal. The maximal decrease is usually observed when the animals are active and after a 25 to 32 per cent loss in body weight has occurred. The cell counts thereafter depend on the nearness of the fatal termination of fast. As the rats weaken, the water intake decreases and the blood becomes more concentrated so that in prolonged fasting, instead

of an anemia being observed, the cell counts and hemoglobin in extreme cases may reach values 50 to 65 per cent above normal. Little relationship between the changes in erythrocytes and weights was noted and, although a mild anemia was observed, the total red cell counts per unit body weight (*C* and *E*) actually were greater than normal, suggesting the development of hydremia.

The median total blood volumes of the series of fasting rats illustrated in figure 2, *E* (*3a* to *f*) ranged from 2.1 at the 2nd day, to 14 per cent at the 12th day with a median for the series of 11 per cent above the control series. The ratio of the blood weight to body weight likewise increased with fasting so that, although a rather marked change in the body weight occurred with the progression of the fast, no comparable change in the blood volume was observed, indicating the development of plethora. The degree of plethora thus developed in fasting, i.e., up to 12 days, seemed to bear little relationship to time as the blood volumes at the 2nd and 12th days were 58 and 57.85 cc. per kilogram body weight. It would seem therefore that in fasting rats the red cell count falls but at a slower rate than in the body weight. This divergence might easily be explained on the basis of hydremia. The blood volume likewise decreases more slowly than the body weight so that there is an actual plethora. The total red cell count decreases but to a smaller extent than either the weight or the cell count per unit blood volume. The corpuscle count shows both a relative and an absolute decrease which increases with the duration of the fast but the extent of the anemia is probably magnified by the simultaneous development of hydremic plethora.

The changes thus observed in acute inanition, i.e., after 10 or more days of fasting are strikingly similar to comparable observations obtained from rats receiving for a prolonged period a vitamin deficient diet (primarily deficient in the B factor), consisting of cooked polished rice and salts.

CONCLUSIONS

The development of inanition in rats during the course of rice disease or in complete fasting is accompanied by a diminution of the red cell count, hemoglobin and the total blood volume. The erythrocytes decrease somewhat less than the body weight, and the ratio of blood weight increases. These changes indicate the development of a hydremic plethora. The degree of change is closely associated with the degree of inanition developed.

Lactose, magnesium carbonate or mineral oil tend to antidote the anemia of inanition. The beneficial action noted was not due to blood concentration but to a diminution of the usual rate of blood destruction.

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STUDIES IN HUMAN PHYSIOLOGY

IV. Vital Capacity, Respiratory Rate and Volume, and Composition of the Expired Air

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In the previous numbers of this series may be found a record of the intra-individual variations of metabolism (1), cardio-vascular conditions (2), and alveolar air and blood-gas capacities (3) of five normal, adult, human subjects who were under observation during the two-year period, February 1925 to February 1927.

The introductory part of the first paper should be consulted for details as to the general plan and routine of the determinations. It must suffice, here, to recall that two of the subjects, A. B. and C. D., were men and were under observation continuously throughout the entire two-year period. The remaining three subjects were women; two of them, E. F. and G. H., served during the first year, February 1925 to February 1926; the third, K. L., was under observation during the second year, from February 1926 to February 1927.

During the first year determinations were made on each of the four subjects, A. B., C. D., E. F., and G. H., approximately once a week; during the second year, on the other hand, each of the subjects, A. B., C. D., and K. L., reported for observation on an average of twice a week.

This report deals with the volume and rate of respiration and the vital capacity of these subjects. Of these, the data in regard to the respiration pertain to the basal condition. Preliminary to the metabolism determinations the subjects were fitted with a Fitz pneumograph (4) which was connected with a recording tambour. The pneumograph was always carefully adjusted so as to cause no annoyance and in fact was unnoticeable. The recording tambour wrote on a smoked kymograph drum to which was also adjusted a time marker and a signal magnet; the latter was connected with a key which was closed and opened by the operator at the beginning and end of the metabolism period. In this way there was obtained a record of the number of respirations during each ten-minute metabolism period; from June of the first year (1925) until the end these were almost

invariably run in duplicate on A. B., C. D., and K. L., and whenever time permitted on E. F. and G. H. When this was the case, in order to tire the subject as little as possible, it was the custom to remove the mouthpiece and nose-clip at the end of the first period so that the breathing might be unobstructed and normal during the ten minutes or so required to make the temperature, barometer and spirometer readings and secure samples of the air in the spirometer. During this intermission the subject remained perfectly quiet and relaxed; and it may be stated that upon resumption of the second determination the same precautions were observed as at the beginning as to rinsing out the spirometer—not merely to remove any traces of atmospheric air that might have entered the connecting tubing but also in order to allow three or four minutes to elapse for the subsidence of any disturbance that might have resulted from the renewed application of the mouthpiece and nose-clip.

During the second year, i.e., from February 1926 to February 1927 and therefore only with subjects A. B., C. D., and K. L., it was customary to secure an additional record of the respiration toward the end of this period of intermission; viz., after the subject had been breathing normally and without any obstruction for several minutes and just before beginning the second metabolism determination. The recording apparatus was situated behind the subject so this record could be obtained without in any way attracting the attention and therefore without any conscious modification of the breathing. The kymograph was speeded up for this record so that the inspiratory and expiratory phases could be accurately measured. For this purpose as many complete respirations were carefully measured as seemed necessary to secure a representative average; ten was the minimum number and then only if the respiration was very regular; if at all irregular, the average was based on twenty-five or more separate measurements.

For both years we have, then, the respiratory rates during the collection of the expired air for the metabolism determinations; and in addition, there is for the second year and for comparison with these, the average inspiration and expiration and rate of the normal respiration, secured under as nearly identical conditions as possible except for the avoidance of any possible modification that might arise from the use of the mouthpiece and nose-clip or other effect that might be caused by breathing into the spirometer.

The tidal and minute volumes are derived from the spirometer readings and the rates of respiration observed during the ten-minute collections of the expired air for the metabolism determinations. In the tables these have been recorded as reduced to standard conditions, 760 mm. and 0°C., and also as recalculated to body temperature, 37°C., and the barometric pressure that prevailed at the time of collection. All of these calculations

as well as all those involved in the metabolism determinations were greatly facilitated by the use of Carpenter's tables (5) and it is an inexcusable negligence that our indebtedness to this extremely helpful aid should have remained unacknowledged until now. Both sets of values have been recorded because of the special interest that attaches to each for certain purposes. The actual volumes of air breathed have perhaps the greatest immediate physiological significance so the statistical analysis has been based on these figures.

It remains to mention a matter of considerable interest in itself and which is of importance also because it is responsible for a sharp dichotomy between the results of the two years. During the first year, February 1925 to February 1926, the side-arm of the "T" tube which connected the subject with the Saad valves, together with the stem of the rubber mouthpiece, constituted an additional dead-space of approximately 50 cc. During the second year both of these were shortened so that when the mouthpiece was in place the subject was breathing almost directly into the free space between the valves and therefore with practically no dead-space increment.

This difference in method should be kept in mind as it will have to be referred to later as an explanation of the different results that were obtained in the two years. It may be said in anticipation that the difference occasioned in this way is quantitative rather than one of kind. The data of the second year, with practically no rebreathing, are obviously more nearly normal than the others; and a comparison of the two, especially when it can be made in the same subjects, as in the cases of A. B. and C. D., who served through both years, throws interesting light on the manner in which the respiration is adjusted in response to the amount of rebreathing involved in the technique of the first year.

The methods of sampling and analyzing the expired air have been described in the first paper of this series and need not be repeated here.

Determination of the vital capacity. It may also be recalled from the previous papers of this series that upon completion of the collection of the expired air for the metabolism determinations, and of obtaining alveolar air samples, the subject arose and went through with the performance necessary for the determination of Schneider's cardio-vascular rating. At the end of this the vital capacity was determined. This was done by having the subject expire, from the standing position, into the 100 liter spirometer which was previously used for the collection of the expired air during the metabolism determinations. In order to make up for the lack of precision with which such a large spirometer can be read, several expirations, properly spaced, were made in the course of a single determination and the average of the three best checks recorded as the day's value.

The temperature of the spirometer and the barometric pressure were

TABLE 1

Statistical constants

FUNCTION	SUBJECT AND YEAR	NUMBER OF OBSERVATIONS	MAXIMUM AND MINIMUM	MODE	ARITHMETICAL MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Vital capacity; (cc.) (37°C. and observed barometer	A. B. (both years)	91	2,800–3,300	3,200	3,118± 9	118	3.8
	C. D. (both years)	91	4,200–4,900	4,600	4,556±10	147	3.2
	E. F. (1925)	37	2,900–3,300	3,200	3,165±15	131	4.1
	G. H. (1925)	24	2,800–3,700	3,100	3,200±29	208	6.5
	K. L. (1926)	50	2,500–3,000	2,700	2,748±13	134	4.9
	Average					148	4.5
Normal respiration:							
Rate per minute	A. B. (1926)	88	9–18	14	13.8±0.1	1.43	10.4
	C. D. (1926)	88	12–18	14	14.0±0.1	1.03	7.4
	K. L. (1926)	84	12–19	15	15.1±0.1	1.27	8.4
	Av.				14.3	1.24	8 7
Inspiration (seconds)	A. B. (1926)	88	1.2–2.3	1.5	1.56±0.02	0.23	14.7
	C. D. (1926)	88	1.3–2.3	1.7	1.77±0.02	0.22	12.4
	K. L. (1926)	84	1.1–2.1	1.4	1.40±0.01	0.16	11.5
	Av.				1.58	0.20	12.9
Expiration (seconds)	A. B. (1926)	88	1.9–4.3	2.9	2.85±0.03	0.39	13.5
	C. D. (1926)	88	2.1–3.2	2.5	2.56±0.02	0.24	9.4
	K. L. (1926)	84	1.9–3.1	2.6	2.61±0.02	0.28	10.6
	Av.				2.67	0.30	11.2
Respiration during collection of expired air for the metabolism determinations:							
Rate per minute	A. B. (1925)	79	7–15	13	11.3±0.1	1.68	14.9
	(1926)	181	8–15	13	12.1±0.1	1.22	10.0
	C. D. (1925)	75	11–16	14	14.0±0.1	0.93	6.7
	(1926)	171	11–14	13	12.8±0.0	0.57	4.5
	E. F. (1925)	65	13–22	16	16.0±0.2	1.90	11.9
	G. H. (1925)	55	14.21	15	15.8±0.1	1.27	8.0
	K. L. (1926)	164	13–16	15	14.6±0.0	0.74	5.1
	Av. 1925				14.3	1.45	10.4
	Av. 1926				13.2	0.84	6.5

TABLE 1—*Concluded*

FUNCTION	SUBJECT AND YEAR	NUMBER OF OBSERVATIONS	MAXIMUM AND MINIMUM	MODE	ARITHMETICAL MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Tidal volume (cc.) (37°C. and observed barometer)	A. B. (1925)	79	320–740	420, 470, 490	487±6	89	18.2
	(1926)	181	330–600	360	401±3	50	12.4
	C. D. (1925)	75	290–490	440	415±4	49	11.9
	(1926)	171	370–470	400	406±1	20	4.9
	E. F. (1925)	65	290–480	410	380±4	49	13.0
	G. H. (1925)	55	300–500	400, 420	404±4	41	10.2
	K. L. (1926)	164	290–380	330	334±1	17	5.2
	Av. 1925				422	57	13.3
	Av. 1926				380	29	5.6
Minute volume (cc.) (37°C. and observed barometer)	A. B. (1925)	79	4,200–6,200	5,500	5,313±30	426	8.0
	(1926)	181	4,300–5,400	4,800	4,755±11	221	4.6
	C. D. (1925)	75	4,300–6,800	6,000, 6,200	5,737±39	531	9.3
	(1926)	171	4,500–5,700	5,200	5,176±13	246	4.7
	E. F. (1925)	65	4,800–7,100	5,600	5,988±46	555	9.3
	G. H. (1925)	55	4,400–8,500	6,300	6,329±62	684	10.8
	K. L. (1926)	164	4,200–5,500	4,700	4,844±15	282	5.8
	Av. 1925				5,842	549	9.4
	Av. 1926				4,925	250	5.0
CO_2 per cent in expired air	A. B. (1925)	79	3.1–4.1	3.6	3.59±0.02	0.22	6.2
	(1926)	181	3.7–4.2	3.8	3.88±0.01	0.11	3.0
	C. D. (1925)	75	3.0–4.5	3.4	3.68±0.03	0.34	9.3
	(1926)	171	3.7–4.4	4.0	3.96±0.01	0.12	2.9
	E. F. (1925)	65	2.7–3.9	3.1	3.25±0.02	0.29	9.0
	G. H. (1925)	55	2.5–4.0	3.0	3.11±0.03	0.33	10.6
	K. L. (1926)	164	3.3–3.9	3.6	3.58±0.01	0.15	4.1
	Av. 1925				3.41	0.32	8.8
	Av. 1926				3.81	0.13	3.3
O_2 per cent in expired air	A. B. (1925)	79	16.1–17.6	17.0	16.91±0.02	0.31	1.8
	(1926)	181	16.1–16.9	16.6	16.53±0.01	0.16	1.0
	C. D. (1925)	75	15.8–17.5	17.0	16.78±0.03	0.39	2.3
	(1926)	171	15.8–16.7	16.4	16.36±0.01	0.20	1.2
	E. F. (1925)	65	16.3–17.8	17.5	17.24±0.03	0.36	1.7
	G. H. (1925)	55	16.1–18.2	17.5, 17.6	17.39±0.04	0.42	2.4
	K. L. (1926)	164	16.0–17.0	16.7	16.56±0.01	0.21	1.3
	Av. 1925				17.08	0.37	2.1
	Av. 1926				16.48	0.19	1.2

noted and from these were calculated the volume of the vital capacity at standard conditions as well as at body temperature and the observed barometric pressure. Both of these sets of values have been recorded because we have never been able to determine from previously published data on the vital capacity to what conditions of temperature and pressure the gas volumes are supposed to apply.

RESULTS. *I. Statistics.* The statistical constants for these data are given in table 1.

1. The effect of rebreathing. The most outstanding feature of table 1 is the marked difference between the statistical values for each of the two years. This can only be attributed to the increased amount of rebreathing which resulted from the enlargement of the dead space during the first year. As might have been expected, the larger the dead space, the greater is the rate and volume of respiration and the oxygen per cent, and the less is the carbon dioxide per cent of the expired air. The only really valid comparisons here are between the mean values for A. B. and C. D. for each of the two years, since they were the only subjects who served under both conditions. Their figures conform to the general averages, however, with the single exception of the respiratory rate of A. B., which is lower during 1925, when the dead space and rebreathing were large, than during 1926 when both were reduced to a minimum.

As a matter of fact it cannot be concluded that the effect of the rebreathing is to increase the respiratory *rate;* for if the rates of A. B., C. D., and K. L. during the collection of the expired air in 1926 are compared with their normal values for the same year it will be seen that in each instance the latter are higher than the former, the averages being 14.3 and 13.2, respectively. This is of interest as indicating that the adjustment to this type of demand for increased respiration is almost entirely effected by variation in the minute and tidal volumes, with compensatory alterations in the composition of the expired air.

Even more striking, however, than the difference in the mean values of the volume and composition of the expired air is the difference in the variability of these functions which results from changes in the volume of the dead space and the amount of rebreathing which it entails. Thus during 1925 when the dead space and rebreathing were relatively large, the standard deviations and the coefficients of variation are, on the average, about twice as great as during the second year when the dead space and rebreathing were reduced to a minimum.

This difference in variability can hardly be attributed to variations in the dead space itself, i.e., within either year, for identically the same apparatus was used throughout each year; and the absolute variations in the size of the dead space were certainly no greater during 1925, when it was large, then during the following year when it was small. Nor can the

lower values of these constants for 1926 be explained as due to the greater number of observations per subject for this year as compared with its predecessor. This might, undoubtedly, play a part in causing such a result; but its inadequacy as even a significant partial explanation is apparent from a comparison of these data with those for the total oxygen consumption and carbon dioxid production as given in the first paper of this series (1). These latter were computed from the very determinations that are being presented here; consequently the number of entries from which the measures of dispersion were calculated are the same in each case. What is found, on making the comparison, is that the standard deviations and coefficients of variation for oxygen comsumption and carbon dioxid production are practically the same for C. D. for both years; and they are practically the same for K. L., with 164 observations as for E. F., with 65, or G. H., with 55; only A. B. shows a slightly lower variability during the second year, but with nothing like the same difference that is found for these respiratory functions. This comparison is very instructive also as showing the relative constancy of the metabolism as compared with the respiration; and the lack of effect which different methods of collection of the expired air, with their varying effects upon the external respiration, have upon the computed metabolic rate; even though the calculation is based upon these same variable respiratory data. This has already been referred to in the first paper of this series as something to be developed in full at this time; and as confirming an observation originally made by Carpenter.

The increased variability of the respiration when the dead space and rebreathing are large must indicate, therefore, a *bona fide* variability in the physiological response to this condition. This will be further illustrated in a later section where it will be shown that the magnitude of the seasonal variation for each of the two years provides additional confirmation of this conclusion.

2. The difference between duplicate determinations. One of the most unexpected results to emerge from this work was evidence as to the instability of the respiratory rate and volume as compared with the other functions for which we have similar duplicate determinations. This variability is shown in the following tabulations:

VARIATIONS OF DUPLICATE OBSERVATIONS FROM THEIR MEANS		NUMBER OF OBSERVATIONS				
Actual difference	Difference as approximate per cent of the mean*	A. B.	C. D.	K. L.	Total	Per cent of the total observations
Respiratory rate						
Respirations per minute	*per cent*					*per cent*
0.0	0	2	3	11	16	6
±0.10–0.25	±1	26	56	51	143	51
0.26–0.50	3	16	30	15	61	22
0.51–0.75	5	15	7	2	24	10
0.76–1.00	7	12			12	4
1.01–1.25	9	6	1	1	8	3
1.26–1.50	11	2			2	1
1.51–1.75	13	8			8	3
1.76–2.00	15	3			3	1
2.01–2.25	17	1			1	
Total		91	107	80	278	
Tidal volume						
cc.	*per cent*					*per cent*
0	0	0	2	7	9	3
±1–5	±1	10	39	38	87	32
6–10	2	9	32	22	63	23
11–15	3	15	14	7	36	13
16–20	4	10	8	3	21	8
21–25	5	10	5	1	16	6
26–30	7	2	3	1	6	2
31–35	8	7			7	3
36–40	10	4			4	1
41–50	11	8			8	3
51–100	19	16			16	6
Total		91	103	79	273	
Minute volume						
cc.	*per cent*					*per cent*
0	0	0	0	0	0	0
±1–25	±0.5	8	6	11	25	9
26–50	1.0	12	16	10	38	14
51–75	1.5	18	16	11	45	16
76–100	2.0	14	13	13	40	15
101–125	2.5	11	16	10	37	14
126–150	3.0	9	8	6	23	8
151–175	3.5	7	7	8	22	8
176–200	4.0	5	4	3	12	5
201–225	4.5	1	6	3	10	4
226–250	5.0	3	6	2	11	4
251–300	6.0		3	2	5	2
301–400	7.0	1			1	
401–500	9.0	2	2		4	1
Total		91	103	79	273	

* Approximate, because the actual deviations from the means which are grouped in the first column have not been divided in each case by the indivdual mean value; instead, the mid-value of the group has been divided by the average for the three subjects which is, in round numbers, 13, for respiratory rate; 400, for tidal volume; and 5000, for the minute volume. Needless to say, this introduces no appreciable error.

If these figures are compared with those of a somewhat similar table which has been given in the first paper of this series (1, p. 619) for the total oxygen consumption, it can easily be seen to what degree the respiration is exceptionally variable; thus, 95 per cent of the duplicate oxygen determinations agree within three per cent or less with their means, whereas this limit includes only 79 per cent of the observations on respiratory rate; and 71 to 76 per cent of the observations on tidal and minute volume, respectively.

Again, the most extreme deviation of any pair of oxygen determinations from their mean is 5.3 per cent (1, p. 618) whereas the extreme deviation for the respiratory rate is 17 per cent; for the tidal volume, 19 per cent; and minute volume, 9 per cent.

And, finally, a comparison of the average variations from the means tells the same story; for these respiratory functions the figures, derived from the preceding tabulations, are as follows:

	AVERAGE DEVIATION OF DUPLICATE DETERMINATIONS FROM THEIR MEANS; PERCENT OF THE MEAN
Respiratory rate	2.8
Tidal volume	3.7
Minute volume	2.4

The corresponding value for the oxygen determinations is 1.2 per cent (1, p. 618); and for the basal pulse rate, 1.8 per cent (2, p. 299).

These relatively large differences between duplicate determinations of the respiratory values are spontaneous and cannot be attributed to any systematic error in so far as we know. For a while it was thought that the first value was consistently higher than the second, perhaps due to a failure to attain a strictly basal state at the beginning of the experiment. That this is not so, is apparent from the following tabulation:

	NUMBER OF OBSERVATIONS IN WHICH THE FIRST DETERMINATION IS	
	Greater than the second	Less than the second
Respiratory rate	149	113
Tidal volume	130	134
Minute volume	138	135

So that, with the exception of the respiratory rate, and even here the difference is not significant enough to be attributed to a systematic error, the second value of a pair of determinations is as likely as the first to be the larger of the two.

Although there is no real proof that it is not so, it seems improbable that these differences are psychic or emotional in origin. At least, the subjects have been unable to recall any difference of mental state even at the close of periods between which the respiratory differences were large.

It may be thought that too much is being made of this matter; perhaps those with a larger experience in these affairs will see nothing unexpected in the occurrence of such variations. Our apology is that to us these data have presented evidence of a degree of variability in fundamental processes, under as nearly identical and basal conditions as it seems possible to reproduce, that was entirely unsuspected. The difference between duplicate metabolism determinations has been attributed to errors of technique. And perhaps it is. But our evidence shows that the basal pulse rate and respiration, in the determination of which the experimental error is small or absent altogether, show, when reduced to comparable bases, an even greater variability than the computed metabolism.

Not exactly in this same catagory yet related to it, is the degree of correlation between the respiratory rates during the duplicate collections of the expired air and the normal rate during the period of intermission between them. Just as the two former may differ considerably from each other, so their average for any one set of determinations shows but a small part of the correlation that might be expected with the normal rate. The coefficients of correlation are as follows:

A. B.	+0.38 ±0.06
C. D.	+0.18 ±0.07
K. L.	+0.39 ±0.06
Average	+0.32

3. The correlation between the different respiratory functions and between them and the metabolism. In addition to the above table showing the correlation between the normal respiratory rate and the average of the rates observed during the collection of the expired air, we add the following to show the correlation between the intra-individual, day-to-day variations of the minute volume and the respiratory rate (during collection of the expired air) and tidal volume:

	COEFFICIENTS OF CORRELATION BETWEEN THE MINUTE VOLUME AND:			
	Tidal volume		Rate of respiration	
	1925	1926	1925	1926
A. B.	+0.27 ±0.08	+0.43 ±0.04	+0.12 ±0.07	−0.14 ±0.05
C. D.	+0.83 ±0.02	+0.56 ±0.03	−0.10 ±0.07	+0.44 ±0.04
E. F.	+0.57 ±0.06		+0.26 ±0.08	
G. H.	+0.89 ±0.02		+0.31 ±0.08	
K. L.		+0 68 ±0.03		+0.42 ±0.04
Average	+0.64	+0.56	+0.15	+0.24

The data are presented separately for each of the two years in this and in the following tables on account of the slight difference in the collection of the expired air which has been referred to so often before. This makes no appreciable difference in these figures; and these are of significance as showing that variations in the minute volume are largely due to alterations of like sign in the depth of the tidal respirations. Indeed, in two instances, C. D., 1925, and A. B., 1926, the correlation between the total ventilation and the rate of respiration is negative; the figures are not large enough to be of any great significance in themselves; but they may suggest an explanation of the fact which has been noted on a previous page, that the respiratory rates during collection of the expired air in 1926 are slightly lower than the corresponding normal rates, during the determination of which there was no possibility of rebreathing, with its consequent augmentation of the total ventilation.

To what degree the respiration depends upon the metabolic rate of the body at the time, as measured by its oxygen consumption and carbon dioxid production, is shown by the following correlations between the intra-individual, day-to-day variations of these functions:

Coefficients of correlation

	OXYGEN CONSUMPTION		CARBON DIOXID PRODUCTION	
	1925	1926	1925	1926
Minute volume:				
A. B.	+0.34 ±0.07	+0.51 ±0.04	+0.36 ±0.06	+0.90 ±0.01
C. D.	+0.38 ±0.06	+0.46 ±0.04	+0.35 ±0.07	+0.74 ±0.02
E. F.	+0.11 ±0.08		+0.50 ±0.06	
G. H.	0.00		+0.37 ±0.08	
K. L.		+0.28 ±0.05		+0.85 ±0.01
Average	+0.21	+0.42	+0.40	+0.83
Tidal volume:				
A. B.	−0.04 ±0.07	+0.19 ±0.05	+0.32 ±0.07	+0.50 ±0.04
C. D.	+0.56 ±0.05	+0.36 ±0.05	+0.28 ±0.07	+0.72 ±0.03
E. F.	−0.22 ±0.08		+0.16 ±0.08	
G. H.	−0.12 ±0.09		+0.24 ±0.09	
K. L.		+0.76 ±0.02		+0.89 ±0.01
Average	+0.05	+0.44	+0.25	+0.70
Rate of respiration:				
A. B.	+0.16 ±0.07	−0.04 ±0.05	−0.17 ±0.07	−0.27 ±0.05
C. D.	−0.13 ±0.08	0.00	−0.06 ±0.08	+0.18 ±0.05
E. F.	+0.44 ±0.07		+0.27 ±0.08	
G. H.	+0.25 ±0.09		+0.22 ±0.09	
K. L.		0.00		+0.15 ±0.05
Average	+0.18	−0.01	+0.06	+0.02

These figures are especially interesting. In the first place they show that the rate of respiration is governed in no way by the metabolic rate. The tidal and minute volumes, on the other hand, are very significantly related to the rate of metabolism, particularly during 1926. This would seem to show that the increased dead space and rebreathing during 1925 introduced variations in the pulmonary ventilation which had no connection with, and obscured its relationship to the metabolic rate.

In this connection the correlation between the pulmonary ventilation and the carbon dioxid production deserves particular notice. All references to the disturbing effect of "auspumpung" on the apparent carbon dioxid production have purposely been deferred until this point. In the first paper of this series it was shown that the non-protein respiratory quotient was more variable than the total metabolic rate; and from this it was concluded that the metabolic materials were more variable than the total metabolic level. Such a conclusion is of course unjustified unless it can be made reasonably certain that the carbon dioxid output during the period of the determinations was free from untoward disturbances, of which "auspumpung" is recognizably the most to be suspected.

The evidence which we have here in regard to the correlation between the carbon dioxid output and the tidal and minute volumes of the respiration seems to be particularly significant in regard to this point. It will be seen that the correlations are unusually high for 1926 and are more than twice as high for this year as for 1925. And it will be easily appreciated that it was during 1925, when the correlation is low, that conditions were most favorable for an abnormal carbon dioxid output. If we consider A. B. and C. D., alone, 1925 included the period of their adjustment to the technique of the determinations; a matter which is well known to favor an unduly high carbon dioxid output, unless the subjects are already somewhat used to the process, as indeed these were. Nevertheless their correlations are highest during the second year after a year of practice and habituation to the apparatus. But more important, 1925 was the year of the abnormally large dead space with the augmented pulmonary ventilation which it produced. This condition, more than any other, would seem to have favored an increased output of carbon dioxid associated with the increased breathing. And yet the fact seems to show that the increased respiration, due to this artificial condition, only served to obscure, in this case as with the oxygen consumption, a more fundamental relationship between the carbon dioxid production and the volume of respiration.

The conclusion seems well grounded, therefore, that the high correlation between the carbon dioxid output and the minute and tidal volumes is not due to an artificially stimulated respiration washing out correspondingly increased amounts of carbon dioxid; on the other hand, the figures must be taken to mean that the fundamental factor in regulating the

volume of the respiration is the rate of carbon dioxid production; which, of course, is merely confirmation, from a hitherto unused source, of a fact that is already well known. The significance of its substantiation from these data, however, lies chiefly in the fact that it insures confidence in our figures for carbon dioxid production and hence for any conclusions based upon them.

Inter-individual correlation between metabolism and respiration: The number of these subjects is too few to prove anything in regard to inter-individual correlations; but with this understanding, the following figures, derived from the intensive study of these five individuals, are suggestive:

SUBJECT	MEAN OXYGEN CONSUMPTION	MEAN VITAL CAPACITY	MEAN MINUTE VOLUME		MEAN TIDAL VOLUME	
			1925	1926	1925	1926
	cc. per minute	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
A. B.	177	3,118	5,313	4,755	487	401
K. L.	181	2,748		4,844		334
E. F.	186	3,165	5,988		380	
G. H.	188	3,200	6,329		404	
C. D.	200	4,556	5,737	5,176	415	406

It may be said at once that there is no evidence of any inter-individual correlation between metabolic rate and the tidal volume. The same is true for the minute volume for 1925; even if the two sexes are considered separately the slopes of the lines connecting the homo-sexual points are too dissimilar to have any meaning. Here as in so many instances before, the artificial stimulation of the respiration by the large dead space of 1925 would appear to have obscured a fundamental relationship; at least we may judge so from the evidence for 1926 which shows a direct proportionality between oxygen consumption and minute volume. It is emphasized again, however, that at best this is very meagre evidence which has value only as a suggestion of possible relationships.

The same is true of the data in regard to oxygen consumption and vital capacity. In order to make anything of it at all, it is necessary to consider the sexes separately. When this is done the lines connecting the points for the men and the points for the women are sufficiently similar in slope to give some value to the suggestion which is thus offered of a positive correlation between the metabolic rate and the vital capacity.

4. Sex differences. Again very tentatively and without wishing to seem to do more than merely describe what can be observed in these data, we call attention to the following figures taken from table 1:

FUNCTION	MEANS			
	1925		1926	
	Men	Women	Men	Women*
Respiratory rate:				
1. During collection of the expired air	12.7	15.9	12.5	14.6
2. Normal			13.9	15.1
Composition of expired air:				
1. Per cent carbon dioxid	3.64	3.18	3.92	3.58
2. Per cent oxygen	16.90	17.32	16.45	16.56

* There was only one woman subject, K. L., during 1926.

In these functions which, unlike the vital capacity and volume of respiration, might be supposed to be independent of body size, it can be seen that these data show a consistent sex difference for both years. Whether or not such a difference would be confirmed on a larger number of subjects is a matter that would seem deserving of future attention in the light of this suggestion.

On firmer ground is the following comparison of the variability shown by the two sexes; these figures also are transposed from table 1 to facilitate comparison.

	STANDARD DEVIATION				COEFFICIENT OF VARIATION			
	1925		1926		1925		1926	
	Men	Women	Men	Woman*	Men	Women	Men	Woman*
Respiratory rate:								
During collection of expired air	1.31	1.59	0.90	0.74	10.8	10.0	7.3	5.1
Normal			1.23	1.27			8.9	8.4
Inspiration			0.23	0.16			13.6	11.5
Expiration			0.32	0.28			11.5	10.6
Tidal volume	69.00	45.00	35.00	17.00	15.1	12.6	8.7	5.2
Minute volume	479.00	620.00	234.00	282.00	8.7	10.1	4.7	5.8
Composition of the expired air:								
CO_2 per cent	0.28	0.31	0.12	0.15	7.8	9.8	3.0	4.1
O_2 per cent	0.35	0.39	0.18	0.21	2.1	2.1	1.1	1.3

	BOTH YEARS		BOTH YEARS	
	Men	Women	Men	Women
Vital capacity	132	158	3.5	5.2

* Only one woman subject, K. L., during 1926.

The data of the two years show consistent differences in spite of the difference in absolute magnitude of the constants and indicate that there is no sex difference in variability in these functions; the average coefficients of variation for the two years are 7.5 and 7.4 for the men and women, respectively.

In conclusion we should like to summarize the data which have been presented in this and the preceding papers of this series, regarding the variability of the different functions studied. These are arranged in the following table in order of increasing coefficients of variation. For the respiratory functions which are dealt with in this report we have used the average coefficients of variability for 1926, since these values are more nearly normal than those for the preceding year.

FUNCTION	AVERAGE COEFFICIENT OF VARIATION
1. Oral temperature	0.5*
2. Oxygen per cent of expired air	1.2*
3. Alveolar oxygen, per cent	3.3
4. Alveolar oxygen, tension	3.5
5. Carbon dioxid per cent of expired air	3.6*
6. Calories per sq. meter per hour	3.8*
7. Total oxygen consumption, cc. per minute	4.0*
8. Vital capacity	4.4*
9. Basal pulse rate	4.7*
10. Basal systolic blood pressure	4.9
11. Alveolar carbon dioxid per cent	5.1*
12. Alveolar carbon dioxid tension	5.2*
13. Total carbon dioxid production, cc. per minute	5.2*
14. Minute volume	5.3*
15. Non-protein respiratory quotient	5.3
16. Non-protein oxygen, cc. per minute	5.8*
17. Standing systolic blood pressure	6.0*
18. Respiratory rate, collection of expired air	6.2
19. Blood carbon dioxid capacity	6.9
20. Tidal volume	7.0
21. Non-protein carbon dioxid, cc. per minute	7.1*
22. Standing pulse rate	7.4*
23. Pulse rate after exercise	7.8
24. Respiratory rate, normal	8.7
25. Blood oxygen capacity	9.5*
26. Duration of expiration	11.2
27. Duration of inspiration	12.9
28. Protein carbon dioxid, cc. per minute	18.3*
29. Protein oxygen, cc. per minute	18.3*

* Coefficient of variation for the women greater than for the men.

This table will be useful for those who are interested in these matters by making readily available a comparison of the relative variability of these functions. Its pertinence in this connection is the summary which it provides of the relative variability of the men and women. In 18 out of the 29 functions studied, or practically two-thirds of the cases, the women have proven to be more variable than the men. Whether this argues for a disturbing effect of menstruation or whether its cause is to be sought for elsewhere cannot be decided at this time. The fact would seem to be important and to deserve further attention in the future.

II. The effect of sleep on the respiration. As has been mentioned in our first and second reports where its effects on the metabolism and basal pulse rate were described, A. B. and C. D. went to sleep during the first of duplicate determinations 30 and 10 times, respectively. The averages for these periods during the first of which the subject slept and during the second of which he was awake, are given in the following table; and for comparison there are included the averages for the first and second periods of an equal number of duplicate determinations from the same times of the year, during both of which the subjects were awake:

SUBJECT	MINUTE VOLUME			TIDAL VOLUME			RATE OF RESPIRATION		
	First	Second	Diff.	First	Second	Diff.	First	Second	Diff.
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*			
A. B.* (W. 30)	3,887	3,886	1	315	340	25	124	116	8
A. B.* (S. 30)	3,838	3,994	156	300	342	42	128	118	10
C. D.* (W. 10)	4,222	4,250	28	326	333	7	130	128	2
C. D.* (S. 10)	4,237	4,398	162	333	339	6	127	130	3

* W, awake during both of two consecutive determinations; S, asleep during the first of two consecutive determinations; the numbers, 30 and 10, are the number of pairs of observations on which the averages are based. By an unfortunate error the number of observations for A. B. has been given in previous reports as 20.

Again we wish to emphasize the caution that these results do not pretend to define the effect of deep sleep. Such dozing as the subjects might do within the ten-minute metabolism period does seem, however, to have had a definite effect in reducing the minute volume; this amounts to 157 and 134 cc., or 4.0 and 3.1 per cent for A. B. and C. D., respectively. This is in line with the reduction in metabolism and pulse rate as described in the first two papers. Whether this reduction in minute volume should be considered as due to reduction in tidal volume, or rate of respiration, or both, is not certain. These data would indicate the possibility of individual differences; both are affected in the case of A. B.; with C. D., on the other hand, the reduction seems to be accomplished entirely by reduced rate of breathing. From the other evidence which has been

TABLE 2

The effect of menstruation; averages of the observations on the women, arranged according to their position in the menstrual cycle

The numbers in parentheses are the number of observations on which each average is based.

FUNCTION	SUBJECT	MENSTRUAL PERIOD	INTERMENSTRUAL PERIOD			
			First week	Second week	Third week	Fourth week and longer
Vital capacity (cc.) (37°C., observed barometer)	E. F.	3,158 (4)	3,185 (13)	3,113 (6)	3,193 (6)	3,282 (8)
	G. H.	2,954 (4)	3,076 (9)	3,013 (5)	3,099 (9)	3,088 (6)
	K. L.	2,733 (4)	2,739 (11)	2,784 (10)	2,712 (10)	2,736 (9)
	Av.	2,949 (12)	3,007 (33)	2,932 (21)	2,967 (25)	2,974 (23)
Respiration during collection of expired air for the metabolism determinations:						
Respiration rate per minute	E. F.	16.7 (9)	16.1 (17)	15.8 (11)	15.7 (13)	15.6 (15)
	G. H.	14.2 (9)	15.4 (14)	15.4 (11)	16.0 (12)	15.9 (9)
	K. L.	14.4 (12)	14.2 (39)	14.7 (36)	14.6 (36)	14.6 (35)
	Av.	15.0 (30)	14.9 (70)	15.0 (58)	15.1 (61)	15.1 (59)
Minute volume (cc.). (37°C., observed barometer)	E. F.	6,083 (9)	5,847 (17)	5,525 (11)	6,032 (13)	6,225 (15)
	G. H.	6,194 (8)	6,117 (15)	6,105 (11)	6,546 (12)	6,784 (9)
	K. L.	4,671 (12)	4,901 (39)	4,715 (36)	5,001 (36)	5,080 (35)
	Av.	5,529 (29)	5,384 (71)	5,132 (58)	5,525 (61)	5,631 (59)
Tidal volume (cc.) (37°C., observed barometer)	E. F.	370 (9)	371 (17)	353 (11)	388 (13)	409 (15)
	G. H.	387 (8)	397 (14)	398 (11)	411 (12)	427 (9)
	K. L.	324 (12)	331 (39)	321 (36)	343 (36)	347 (35)
	Av.	356 (29)	354 (70)	342 (58)	366 (61)	375 (59)
Carbon dioxid per cent of expired air	E. F.	3.21 (9)	3.26 (17)	3.44 (11)	3.27 (13)	3.14 (15)
	G. H.	3.18 (8)	3.15 (15)	3.19 (11)	3.04 (12)	2.93 (9)
	K. L.	3.59 (12)	3.66 (39)	3.65 (35)	3.49 (36)	3.48 (35)
	Av.	3.36 (29)	3.46 (71)	3.52 (57)	3.35 (61)	3.31 (59)
Oxygen, per cent of expired air	E. F.	17.29 (9)	17.20 (17)	16.94 (11)	17.22 (13)	17.36 (15)
	G. H.	17.26 (8)	17.30 (15)	17.31 (11)	17.44 (12)	17.58 (9)
	K. L.	16.57 (12)	16.47 (39)	16.43 (35)	16.64 (36)	16.69 (35)
	Av.	16.98 (29)	16.82 (71)	16.70 (57)	16.92 (61)	16.99 (59)

given in this paper that adjustments of pulmonary ventilation usually involve variations of tidal volume to a greater extent than of rate, this result for C. D. may be considered less typical than the other and due, perhaps, to the smaller number of determinations on which his averages are based.

III. The effect of menstruation. In table 2 the data for the three women are arranged according to their incidence in relation to the menstrual cycle.

It may be said at once that there is no evidence for an effect of menstruation on either the vital capacity or rate of respiration. On the other hand, the minute and tidal volumes and the composition of the expired air do seem to be definitely affected; and the grand averages for these functions, which are shown graphically in figure 1, are based on sufficiently concurrent testimony to have a strong validity. Thus all of the subjects are alike in showing a sharp rise in the tidal and minute volumes, which begins in the middle of the inter-menstrual period and culminates in the week just preceding the onset of menstruation; the oxygen percentage of the expired air follows a similar rise during the same period, while the carbon dioxid percentage falls reciprocally.

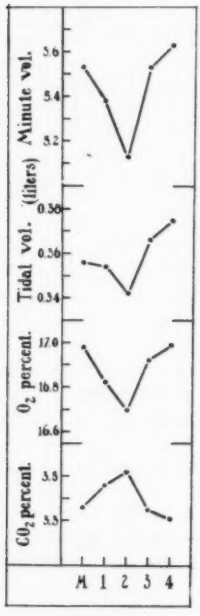

Fig. 1. The effect of menstruation; grand averages from the data of table 2. *M*, *1*, *2*, *3*, and *4* are respectively the menstrual period and the first, second, etc., weeks of the inter-menstrual period.

So much is very definite; on the other hand, just what values to assign to the menstrual period itself and the week immediately following, in relation to this latter part of the curve, is not so clear. From the confidence which we have in the data for K. L. (1, p. 624) and the fact that in these functions her testimony is corroborated by that of G. H., we are inclined to believe that the average values for the menstrual period and the week following are rendered too high by the aberrant values of E. F. during this time. This is more particularly true of the minute volume; in the cases of the tidal volume there is so little divergence among the three subjects, even in this part of the period, that it is difficult to doubt the substantial accuracy of the average curve throughout; and the same is true for the data in regard to the composition of the expired air.

Our conclusion, then, would be that the tidal and minute volumes are perhaps slightly lower just after menstruation than during the period itself; and that they rise rapidly during the latter part of the inter-

menstrual period to their largest values just before the beginning of the next menstruation. The oxygen percentage of the expired air follows a similar course and is followed in an inverse relationship by the percentage of carbon dioxid.

It is worth noting that the curves for minute and tidal volumes, both individually and as grand averages, are markedly similar to those for oxygen consumption and carbon dioxid production, as given in our first report (1, p. 623). This is significant in the light of the rather high correlation which, on a previous page, was shown to exist between these variables for the data as a whole. Thus their correspondence during the menstrual cycle would seem to constitute a strong presumption against the possibility of these variations, either of metabolism or pulmonary ventilation, being the result of chance.

Since we may not have occasion to refer to this matter in detail again it may be permissible to refer here to further consistencies of interrelationship which give added credence to the possibility of genuine menstrual variation. Thus in addition to the correlation which has just been referred to between the variations of metabolism and pulmonary ventilation throughout the menstrual cycles of these subjects, it may be seen by reference to our second and third papers that further consistent correlations are to be found in the menstrual variations of the basal pulse rate and composition of the alveolar air.

For example, in the light of the rather high correlation which has been shown to exist for the data as a whole between the basal pulse rate and the rate of metabolism (2, p. 302) it could be expected that during the first part of the inter-menstrual period, when the metabolism is low, the pulse rate should also be low; and conversely, during the latter part of the period, when the metabolic rate is highest, the pulse rate should be highest. Such, in fact, proves to be the case.

And finally, as was shown in the third report, the alveolar carbon dioxid is lowest just preceding menstruation and rises to a maximum toward the middle of the inter-menstrual period; in other words, the alveolar carbon dioxid concentration varies inversely with the pulmonary ventilation during the menstrual cycle. At the same time it was shown that the blood carbon dioxid *capacity* varied in the same manner as the alveolar carbon dioxid concentration. This would seem to imply periodic variations in the blood alkali reserve; when this is at its highest value toward the end of the first half of the inter-menstrual period not only would a higher alveolar carbon dioxid concentration be tolerated but it would be necessary in order to provide the usual stimulus to respiration. And when it is remembered that the metabolic rate, i.e., the rate of carbon dioxid production, is lowest at just this time, the decreased pulmonary ventilation follows as a logical consequence. In like manner the reciprocal variations during the latter part of the menstrual cycle may be explained in a similar way.

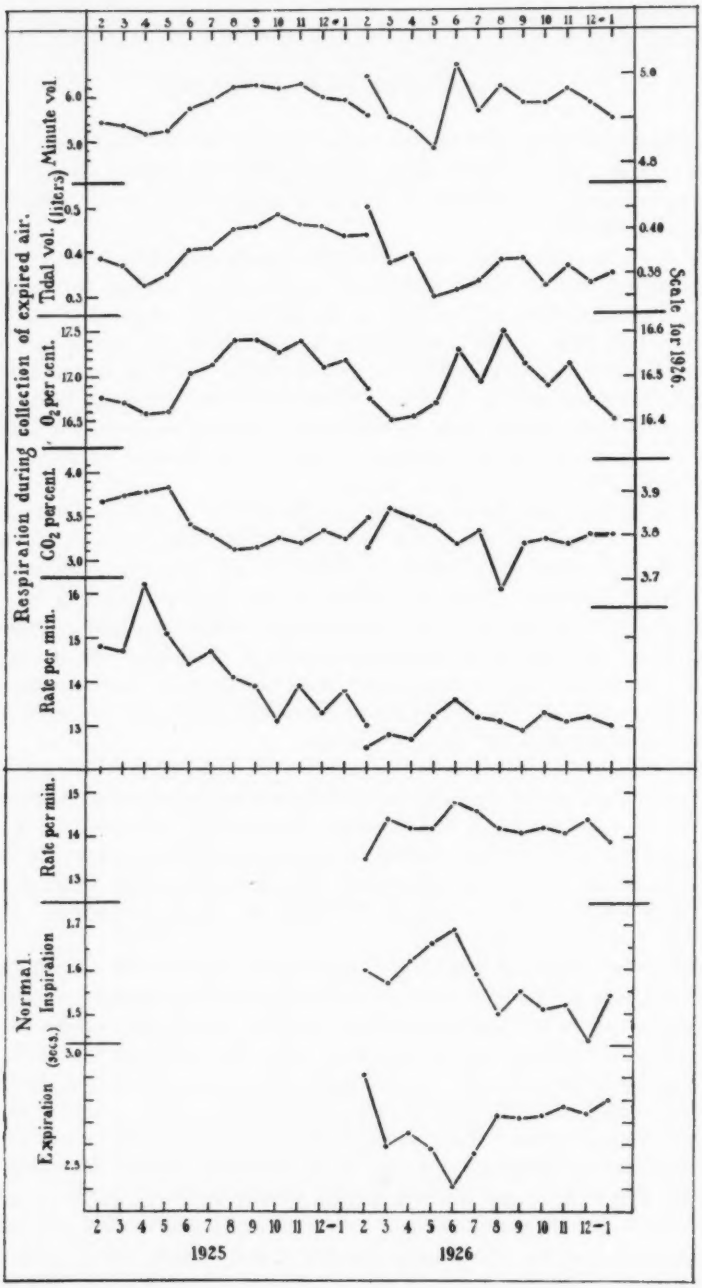

Fig. 2. Seasonal variation; grand averages for each month from the data of table 3. It will be noted that the curves for 1926–7 for the tidal and minute volumes and the composition of the expired air are plotted on a much larger scale than during 1925; for explanation see the text.

It is not intended that this explanation should be accepted as final. All that it is desired to do is to point out the seemingly coördinated variation of these different functions; whatever the final explanation of it may prove to be, the temporal correlation of so many variables would seem to indicate that we are dealing here with a genuine physiological disturbance and not with fortuitous variations.

IV. Seasonal periodicity. The monthly averages upon which the conclusions of this section rest are given in table 3; the grand averages computed therefrom are shown, with the exception of the vital capacity, in figure 2.

Neither the vital capacity nor the rate of respiration appear to us to have any seasonal variation. This does not mean that the vital capacity is invariable; in the case of A. B. it rises gradually to a maximum during the first year and subsides to its original value at the end of the second; with C. D. it rises gradually throughout the two year period; and each of the other subjects shows individual peculiarities which make it impossible to speak of anything like a common seasonal variation for this function.

We do not believe, either, that it is possible to make out a seasonal variation in the case of the respiratory rate; the average curves are reproduced in figure 2 to show the correspondence in the fortuitous fluctuations of the normal rate and the rate during the collection of the expired air during the second year. During this year there does seem to be a maximum during the summer; but the average effect is small and the individuals are not at all concurrent; neither are the curves for the rate during the collection of the expired air at all alike for the two years.

Another reason for producing the curve for normal respiratory rate is for the purpose of contrasting it with the curves for the lengths of the inspiratory and expiratory phases. These components of the respiratory act show a most definite seasonal variation in length; and, curiously enough, in such an exactly reciprocal manner as to leave the total length apparently unaffected, as was remarked above. Unfortunately we have these data for only one year; but they inspire confidence on account of the substantial agreement among the three subjects and the clear-cut nature of the result.

The evidence for seasonal variation of the minute and tidal volumes and the composition of the expired air is the most definite, uniform and conclusive of any that we have for any of the functions that we have studied; the precision with which the observations on the different subjects agree as to the magnitude and time of incidence of the maximum and minimum effects makes the average curves of figure 2 unusually trustworthy. From them it will be seen that the tidal and minute volumes are lowest in the spring and increase uniformly to a maximum in the late summer and fall; the oxygen percentage of the expired air follows an

TABLE 3

Monthly averages

SUBJECT	DATE	VITAL CAPACITY		RESPIRATION										
				Normal			During collection of expired air							
								Minute volume		Tidal volume		Composition of the expired air		
		0°C.—760 mm.	37°C.—observed barometer	Inspiration	Expiration	Rate per minute	Rate per minute	0°C.—760 mm.	37°C.—observed barometer	0°C.—760 mm.	37°C.—observed barometer	Per cent CO_2	Per cent O_2	Number of observations
		cc.	cc.	seconds	seconds			cc.	cc.	cc.	cc.			
E.F.	1925 Feb.	2,634	3,247				18.1	4,449	5,526	246	306	3.57	16.78	4
	Mar.	2,584	3,210				15.7	4,272	5,286	273	337	3.71	16.65	4
	Apr.	2,598	3,222				18.0	4,929	6,111	274	340	3.31	17.22	5
	May	2,621	3,239				17.7	4,405	5,433	249	308	3.68	16.77	3
	June	2,498	3,073				15.8	4,635	5,698	293	360	3.26	17.05	8
	July	2,519	3,108				16.0	4,819	5,952	301	372	3.17	17.23	10
	Aug.	2,600	3,195				16.7	5,498	6,755	329	404	2.93	17.61	5
	Sep.	2,596	3,204				14.9	5,077	6,255	341	420	3.06	17.49	7
	Oct	2,525	3,098				15.2	5,303	6,502	351	430	3.10	17.54	5
	Nov.	2,548	3,152				14.9	5,197	6,434	348	430	3.06	17.58	3
	Dec.	2,591	3,197				13.9	4,711	5,817	341	420	3.29	17.14	5
	1926 Jan.	2,597	3,166				14.1	4,903	6,019	348	427	3.12	17.33	4
	Feb.						14.2	4,791	5,869	340	417	3.25	17.16	2
G. H.	1925 Feb.						16.5	5,180	6,349	316	387	3.24	17.22	2
	Mar.						15.3	4,733	5,797	309	379	3.50	16.84	4
	Apr.						16.5	4,249	5,255	259	318	3.65	16.65	4
	May	2,716	3,334				15.6	4,731	5,824	304	374	3.58	16.89	5
	June	2,573	3,154				15.7	5,009	6,160	319	392	3.08	17.37	7
	July	2,434	3,002				16.9	5,507	6,794	325	401	2.69	17.64	8
	Aug.	2,710	3,343				15.1	5,196	6,409	345	425	2.93	17.59	3
	Sep.	2,509	3,083				15.1	5,544	6,834	366	451	2.85	17.71	4
	Oct.	2,556	3,159				14.8	5,383	6,658	366	452	2.98	17.61	4
	Nov.	2,857	3,483				15.7	5,634	6,873	360	440	2.77	17.79	2
	Dec.	2,706	3,318				15.5	5,495	6,758	354	436	2.91	17.57	4
	1926 Jan.	2,633	3,263				15.3	5,074	6,289	332	412	2.91	17.54	6
	Feb.	2,428	2,994				15.0	5,212	6,434	348	429	2.93	17.44	2
K. L.	1926 Feb.	2,255	2,760	1.50	2.37	15.5	14.9	3,980	4,926	266	330	3.61	16.46	6
	Mar.	2,282	2,839	1.34	2.58	15.2	13.9	3,922	4,848	282	348	3.72	16.32	18
	Apr.	2,326	2,865	1.42	2.43	15.6	14.3	3,941	4,844	276	339	3.70	16.41	18
	May	2,243	2,762	1.42	2.60	15.0	14.8	3,949	4,854	268	329	3.57	16.56	16
	June	2,325	2,868	1.42	2.26	16.5	15.3	4,141	5,114	270	333	3.45	16.77	18
	July	2,216	2,714	1.31	2.43	16.1	14.6	3,910	4,790	268	328	3.58	16.51	12
	Aug.	2,277	2,793	1.37	2.73	14.7	14.8	3,955	4,847	268	328	3.47	16.72	10

TABLE 3—*Continued*

SUBJECT	DATE	VITAL CAPACITY		RESPIRATION										
				Normal			During collection of expired air							
								Minute volume		Tidal volume		Composition of the expired air		
		0°C.—760 mm.	37°C.—observed barometer	Inspiration	Expiration	Rate per minute	Rate per minute	0°C.—760 mm.	37°C.—observed barometer	0°C.—760 mm.	37°C.—observed barometer	Per cent CO_2	Per cent O_2	Number of observations
		cc.	*cc.*	*seconds*	*seconds*			*cc.*	*cc.*	*cc.*	*cc.*			
K. L.	1926 Sep.	2,196	2,698	1.50	2.77	14.1	14.5	3,852	4,716	266	326	3.49	16.65	16
	Oct.	2,198	2,728	1.39	2.78	14.4	14.9	3,853	4,769	258	319	3.56	16.55	12
	Nov.	2,151	2,641	1.33	2.73	14.8	14.6	3,984	4,893	273	335	3.47	16.72	17
	Dec.	2,135	2,619	1.34	2.81	14.6	13.9	3,915	4,808	282	346	3.60	16.55	13
	1927 Jan.	2,111	2,602	1.51	2.97	13.4	13.7	3,840	4,756	281	348	3.68	16.33	6
A. B.	1925 Feb.	2,384	2,914				9.4	3,911	4,780	419	512	3.84	16.62	3
	Mar.	2,430	2,986				12.5	4,196	5,155	340	417	3.68	16.81	4
	Apr.	2,422	2,963				13.9	3,981	4,869	289	353	3.86	16.53	4
	May	2,432	2,987				13.1	3,974	4,849	304	372	3.82	16.59	4
	June	2,462	3,028				12.3	4,606	5,666	376	462	3.48	17.06	11
	July	2,509	3,097				11.4	4,284	5,288	380	469	3.51	16.96	9
	Aug.	2,632	3,222				10.9	4,632	5,668	435	532	3.29	17.29	6
	Sep.	2,665	3,273				11.3	4,668	5,720	420	515	3.40	17.19	7
	Oct.	2,610	3,209				8.4	4,415	5,430	498	613	3.59	16.98	9
	Nov.	2,641	3,220				11.1	4,594	5,599	428	521	3.53	17.13	9
	Dec.	2,597	3,205				10.0	4,287	5,296	431	532	3.67	16.82	5
	1926 Jan.	2,622	3,225				12.6	4,395	5,408	350	431	3.44	16.95	8
	Feb.	2,574	3,186	1.66	3.52	11.7	10.0	3,779	4,668	386	477	3.86	16.42	11
	Mar.	2,543	3,143	1.63	2.76	13.7	11.6	3,686	4,554	322	398	3.92	16.41	20
	Apr.	2,581	3,185	1.56	3.07	13.2	11.1	3,885	4,782	356	438	3.86	16.48	16
	May	2,550	3,161	1.53	2.80	13.8	12.2	3,753	4,639	308	380	3.86	16.47	17
	June	2,540	3,132	1.66	2.61	14.2	12.4	3,810	4,701	309	381	3.97	16.47	18
	July	2,540	3,123	1.71	2.66	13.8	12.1	3,894	4,786	324	398	3.97	16.54	14
	Aug.	2,511	3,082	1.55	2.81	13.8	11.5	3,902	4,788	343	420	3.75	16.66	11
	Sep.	2,488	3,050	1.42	2.79	14.4	11.7	3,982	4,878	341	422	3.85	16.63	18
	Oct.	2,426	3,007	1.46	2.84	14.0	12.2	3,941	4,872	327	404	3.86	16.55	16
	Nov.	2,531	3,101	1.53	2.85	13.8	12.2	3,937	4,834	329	404	3.87	16.59	18
	Dec.	2,455	3,016	1.39	2.75	14.5	13.0	3,900	4,793	301	370	3.81	16.52	14
	1927 Jan.	2,432	2,996	1.43	2.76	14.3	12.8	3,936	4,843	309	381	3.80	16.58	8
C. D.	1925 Feb.	3,525	4,329				15.1	4,158	5,107	276	339	4.04	16.41	2
	Mar.	3,592	4,385				15.4	4,369	5,334	283	346	4.01	16.52	5
	Apr.	3,623	4,424				14.8	3,748	4,574	253	309	4.32	16.04	4
	May	3,649	4,494				14.1	3,926	4,836	279	344	4.25	16.15	4
	June	3,481	4,286				13.9	4,452	5,481	320	394	3.80	16.69	10

TABLE 3—*Concluded*

SUBJECT	DATE	VITAL CAPACITY		RESPIRATION										
				Normal			During collection of expired air							
								Minute volume		Tidal volume		Composition of the expired air		
		0°C.—760 mm.	37°C.—observed barometer	Inspiration	Expiration	Rate per minute	Rate per minute	0°C.—760 mm.	37°C.—observed barometer	0°C.—760 mm.	37°C.—observed barometer	Per cent CO_2	Per cent O_2	Number of observations
		cc.	*cc.*	*seconds*	*seconds*			*cc.*	*cc.*	*cc.*	*cc.*			
C. D.	1925 July	3,500	4,307				14.5	4,611	5,674	318	391	3.76	16.73	8
	Aug.	3,728	4,573				13.8	4,942	6,065	358	439	3.32	17.13	5
	Sep.	3,785	4,645				14.1	5,096	6,257	361	443	3.29	17.24	9
	Oct.	3,751	4,638				13.9	5,007	6,190	362	448	3.37	17.00	8
	Nov.	3,695	4,577				13.7	5,052	6,235	371	458	3.42	17.10	7
	Dec.	3,678	4,549				13.6	4,927	6,090	362	448	3.50	16.95	7
	1926 Jan.	3,642	4,492				13.2	4,907	6,020	373	460	3.52	16.97	7
	Feb.	3,592	4,417	1.65	2.83	13.4	12.7	4,059	5,387	344	426	3.83	16.46	10
	Mar.	3,659	4,514	1.75	2.43	14.4	13.0	4,298	5,315	332	410	3.94	16.45	18
	Apr.	3,765	4,641	1.89	2.44	13.9	12.8	4,072	5,009	316	389	3.97	16.35	18
	May	3,773	4,633	2.04	2.35	13.7	12.6	4,096	5,034	325	400	4.02	16.29	16
	June	3,732	4,585	1.99	2.36	13.8	13.0	4,256	5,255	328	404	3.93	16.43	17
	July	3,754	4,620	1.74	2.60	14.0	12.8	4,207	5,173	329	404	3.87	16.41	18
	Aug.	3,806	4,666	1.58	2.66	14.2	12.9	4,312	5,284	335	411	3.83	16.41	10
	Sep.	3,866	4,731	1.73	2.59	13.9	12.6	4,267	5,215	337	412	4.00	16.31	14
	Oct.	3,846	4,735	1.69	2.57	14.2	12.9	4,200	5,168	326	401	3.96	16.33	16
	Nov.	3,805	4,691	1.71	2.73	13.6	12.5	4,206	5,171	337	414	4.01	16.29	16
	Dec.	3,798	4,645	1.58	2.66	14.2	12.7	4,271	5,211	337	411	3.99	16.27	14
	1927 Jan.	3,796	4,690	1.68	2.66	13.9	12.4	4,137	5,098	333	411	3.91	16.29	8

exactly parallel course; and the carbon dioxid percentage varies in an exactly inverse relationship to these.

The curves for minute and tidal volumes of figure 2 are plotted from the gas volumes as recalculated to body temperature, 37°C., at the observed barometric pressures; a comparison of the two sets of data as recorded individually in table 3 will show, however, that the same variation is to be observed after they have been reduced to a common basis of comparison, viz., 0°C., and 760 mm. pressure. The variation is therefore of actual physiological significance and cannot be attributed to fortuitous variations in the measurement of the gas volumes.

An insight into the meaning of this variation may be derived from a comparison of the data for each of the two years. It will be noted that the curves for 1926 (fig. 2) are plotted on a much larger scale than those

of the first year; had they been plotted on the same scale they would be practically straight lines. This magnification accentuates the irregularities and accounts for the angularities of the curves for 1926 as compared with the smoothness of those of 1925; it is indispensable, however, for revealing the essential similarity in kind of the data for the two years.

This difference in absolute magnitude of the effect in the two years must be due to the relatively large dead space and rebreathing which resulted from the apparatus used during 1925 and which has had to be referred to so often before as imposing peculiarities of degree upon the data for this year. From this it may be inferred that normal, natural breathing, with no artificial augmentation of the dead space, which we closely approximated but did not completely achieve during 1926, would probably show very little or no seasonal variation in the volume of the pulmonary ventilation. We are therefore indebted to what we long considered an unhappy technical blunder for revealing a factor which without it might easily have remained unrecognized. For it was only in consequence of the very apparent results of the first year that we were led to inspect the data of the second under sufficient magnification to discover its similar seasonal variation in miniature.

The conclusion from this must be that the sensitivity of the respiratory center changes with the time of year, being least in the spring and greatest in the late summer and early fall. The variations in the pulmonary ventilation which have been observed are therefore measures of the response of the center at different times to a constant stimulus; this stimulus, during these determinations, being the amount of carbon dioxid in the inspired air; which, in turn, was determined by the size of the dead space in the apparatus used for collection of the expired air. Since this was large in 1925 the response of the center was large, a fact which was referred to earlier as evident in the mean values and degree of dispersion of the data for this year; being small in 1926, the response was also smaller, as was again corroborated by the statistical analysis of the previous section.

In the main, this corroborates the conclusion arrived at by Lindhard in the painstaking work (6) to which reference has already been so often made. Although we have been unable to confirm his findings in regard to the seasonal variations of metabolism (1), alveolar carbon dioxid (3), or respiratory rate (below, this paper), we are in agreement as to an increased sensitivity of the respiratory center during the warmer parts of the year, as evidenced by maximal pulmonary ventilation at this time. Lindhard makes no mention, however, of the marked spring depression which is so pronounced in our records.

Again attention may be called to the fact that these variations in pulmonary ventilation seem to be effected entirely by variations in depth rather than in rate of respiration. Thus the latter not only fails to show

any corresponding seasonal variation, but if we refer back to table 1 it can be seen that there is less difference between the statistical values for respiratory rate for the two years than there is for the other components of the respiration. Not only that, but the measures of dispersion and, more particularly, the maxima and minima, the modes and the arithmetical means are of the same order of magnitude for the normal rate as for the rates during collection of the expired air, either for 1925, with the large dead space and augmented ventilation, or for 1926 when these were more nearly normal. The same thing has also been noticed in connection with menstruation where we were unable to find any consistent variation in rate although the volumes and composition of the expired air were quite evidently affected. The only exception to the rule was in the case of the effect of sleep on the respiration of C. D; here the decreased minute volume could only be attributed to change in rate. But this, as already pointed out, may easily be in error on account of the small number of observations involved; and particularly so, since with A. B. the effect was, again, due largely to variation in tidal volume. Thus although the respiratory rate has been shown to be quite unstable, the average deviation of duplicate determinations from their means and the coefficients of variation both being large, this variability would seem to be spontaneous and fortuitous; and, at least under these basal conditions, quite unrelated to the adaptive alterations in pulmonary ventilation which we have been considering.

In conclusion it may be mentioned that the seasonal variation in composition of the expired air seems to be such as to prevent the variation in pulmonary ventilation from obscuring the true gaseous exchange. Reference to our first paper will show that the metabolic rate, whether measured by oxygen consumption or carbon dioxid production, varied within almost exactly the same limits during each of the two years of this study. This, we may suppose with good reason, is quite as it should be. Such a result could not have been derived from these respiratory data, however, unless the variations in pulmonary ventilation, which were roughly twice as great in 1925 as in 1926, had been very accurately compensated for by changes in the composition of the expired air. The consistent interrelationship of all of these variables would seem, therefore, to justify a high degree of confidence in these results for the data as a whole.

By contrast, however, it must be remarked as a seeming defect in the evidence for *seasonal* variation that there is lacking, here, the precise correlation of all the functions which appear to be involved, such as made the picture of menstrual variation so clear-cut and decisive. The most unequivocal examples of seasonal variation from among all of our data are provided by the basal pulse rate which was described in our second report and the pulmonary ventilation given here. These are both very

little liable to falsification through errors of technique; and for both of them there is unanimous agreement among the subjects of this group. But we find that the basal pulse rate is lowest during the summer; whereas the minute and tidal volumes are just as definitely lowest in the spring. The significance of this disagreement becomes apparent when it is attempted to reconcile each of these with the metabolic rate. This has been shown in these papers to have about the same degree of high correlation with both the basal pulse rate and the minute volume for the data as a whole. But insofar as the average effect is concerned the metabolic rate is definitely lowest in the summer, i.e., in its seasonal variation it satisfies the expected correlation with the pulse rate, while violating the other.

It is true that the evidence for a summer depression of the metabolic rate was not unanimous; with C. D., the rate was definitely lowest in the spring; and with A. B., there was in both years a pronounced spring depression. But these individual differences hold no solution of the problem for there were no exceptions to the variations in pulse rate and pulmonary ventilation; and therefore neither individually nor as averages can the expected correlations all be satisfied.

This may mean that what we have called seasonal variations are merely coincident, chance fluctuations; this is all but impossible to believe of evidence as little subject to experimental error, as definite, and as consistently shown by all of the subjects as the variations in pulse rate and minute volume; and while it would not be so difficult to suspect the metabolism data, the mere fact that such definite variations are observable in such fundamental processes as the pulse rate and pulmonary ventilation would make a variation in metabolism seem very reasonble. It may be suggested that the failure to obtain as consistent correlations among the functions seeming to show a seasonal variation as was obtained, for example, in the menstrual cycle, is due to the temporal dispersion of the data which gives opportunity for the play of disturbing factors that do not have time to make themselves felt in periods of shorter duration.

Such a conclusion is altogether too vague to be regarded as a happy ending to this subject. It will serve, however, to give point to our hope that no one will ever accuse us of having pretended to have settled the possibility or exact nature of seasonal variation. Our work was done as carefully as it seemed possible to do it; and our main purpose has been to describe as accurately and with as little bias as possible such results as the data show. But it is thoroughly realized that in the case of a subject as large as that of seasonal periodicity, where an entire year is required for the completion of a single experiment, not only time but even more rigorously controlled determinations will be required to secure enough and adequate data for finally settling this question.

SUMMARY

This is the fourth of a series of reports in which we have described the intra-individual variations of weight, temperature and metabolism (1), pulse rate and systolic blood pressure (2), and the composition of the alveolar air and blood gas capacity (3) of five normal, adult human subjects who were under observation from February 1925 to February 1927.

This paper deals with the variations in vital capacity, the rate and volume of the respiration and the composition of the expired air.

For each of these functions are given the statistical constants defining the modes and means and the extent and degree of variability. In addition we have calculated the degree of correlation between the rate and minute and tidal volumes of the respiration; and between these and the oxygen consumption and carbon dioxid production.

There is an unusually high correlation between the volume of pulmonary ventilation and the carbon dioxid output; reasons are given for believing that this is not due to increased breathing washing out increased amounts of carbon dioxid, but is an expression of the fundamental control of pulmonary ventilation by the rate of carbon dioxid production.

A sharp dichotomy separates the results of the two years as regards the minute and tidal volumes and the composition of the expired air; this was due to the dead space in the apparatus for collecting the expired air being large (about 50 cc.) during the first year and small or practically negligible during the second. The large dead space was associated not only with large absolute values for these functions, but occasioned, also, a much greater degree of variability.

Going to sleep (dozing) during the collection of the expired air definitely decreases the minute volume 3 to 4 per cent; it is not clear to what extent this is due to variations of rate or of tidal volume; though other evidence would seem to indicate that the latter is probably most involved.

Menstruation cannot be seen to have any effect on the vital capacity nor the rate of respiration; on the other hand the volume of pulmonary ventilation and the composition of the expired air are definitely affected. These variations are so concisely illustrated in figure 1 that they need not be repeated here. In addition it is shown that the menstrual variations in metabolism, pulse rate, composition of the alveolar air and blood gas capacity which have been described in the previous reports may be consistently correlated with the changes described here.

Neither the vital capacity nor the respiratory rate can be seen to have a seasonal variation; the tidal and minute volumes and the composition of the expired air, however, show the most definite and uniform variation of any of the functions we have studied except the basal pulse rate; these are so clearly shown in figure 2 that they need not be described again. In addition, attention is called to the difficulty of correlating the seasonal

variations of metabolism, pulse rate and the ventilation which these data seem to show as indicating the need for much further work before this matter can be considered settled.

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PHYSIOLOGICAL VARIATIONS IN THE CARDIAC OUTPUT OF MAN

IV. The Effect of Psychic Disturbances on the Cardiac Output, Pulse, Blood Pressure, and Oxygen Consumption of Man

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That psychic disturbances cause marked rises in the pulse and blood pressure of man is of common knowledge (Weinberg, 1923; Rihl, 1926) and it has often been assumed (Lindhard, 1915; Collet and Liljestrand, 1924) that these vascular changes are accompanied by an increased cardiac output although no direct experimental proof of the latter fact has been presented. The marked variations observed in a series of determinations of the cardiac output, made on the same individual, have, therefore, often been attributed to various assumed psychic factors. The present investigation aimed to determine the effect, if any, of such psychic disturbances on the cardiac output. This problem is of considerable practical importance. Its solution would indicate to what extent one must avoid psychic disturbances in determinations of the cardiac output and also determine to what extent one may attribute observed changes in cardiac output to such disturbances. A knowledge of the changes in the cardiac output accompanying psychic disturbances would also help to elucidate the problem of the physiological changes occurring in the vascular system and the relative importance of vasomotor and cardiac effects during such disturbances.

Methods. The method employed for determining the cardiac output was that utilized in the preceding papers of this series (Grollman, 1929). The subjects were all normal individuals in the third decade of life. The experiments were done in the basal condition and at least 12 hours after the ingestion of any food or fluid. After the preliminary resting period of 40 minutes or more, two series of determinations were made to obtain the resting basal values. The subject was then subjected to some psychic disturbance and the determinations were repeated. In no case did the subjects have any knowledge of the purpose of the experiment, considering all of the experiments as mere repetitions of the basal determinations. After the completion of the experiment, its purpose was explained to the subject who was then requested to state his subjective reactions to the psychic disturbance.

RESULTS. The results of a series of 9 experiments are given in table 1. The first four subjects, W., H., B., and J., were medical students, working at the time of these determinations, in the physiological laboratory. After the preliminary rest period, two duplicate determinations were made to obtain the basal values. These agreed in every case to 0.1 liter and the average value of the 2 determinations is given in the column marked "Basal" of table 1. After the basal determination, the professor in charge of the department[1] then entered the laboratory and accused the subject,

TABLE 1

The effect of psychic disturbances on the pulse, blood pressure, oxygen consumption, and cardiac output of man

SUBJECT	BASAL					PSYCHICALLY DISTURBED				
	Pulse	Blood pressure	Oxygen consumption	Arteriovenous oxygen difference	Cardiac output	Pulse	Blood pressure	Oxygen consumption	Arteriovenous oxygen difference	Cardiac output
			cc. per minute	*cc. per liter*	*cc. per minute*			*cc. per minute*	*cc. per liter*	*liters per minute*
W.	50	104/69	226	60	3.77	58	119/69	240	54	4.44
						50	109/70		62	3.87
H.	60	103/70	230	58	3.97	62	112/80	250	54	4.63
						66	122/86		51	4.90
B.	70	103/65	245	58	4.22	70	105/68	245	56	4.38
J.	60	115/80	210	60	3.50	62	120/85	220	58	3.80
C.	60	97/68	171	63	2.71	68	106/68	180	59	3.05
E.	60	100/71	205	60	3.42	70	120/75	215	50	4.30
G.	60		223	61	3.66	70		240	53	4.53
G.	60	105/70	223	60	3.72	60	105/70	224	61	3.67
C.	60	98/67	170	62	2.74	60	98/68	170	61	2.79

upon whom the determinations were being made, of laxity in his work in the physiology course. A second series of determinations was then carried out in which the pulse, blood pressure, arterio-venous oxygen difference, and the oxygen consumption were determined, followed by another determination of the pulse, blood pressure, and arterio-venous oxygen difference. The single determination of the oxygen consumption was used in calculating the cardiac outputs corresponding to the two arterio-venous oxygen differences.

Subject W. showed resentment at the disparagement of his work.

[1] I am indebted to Professor E. K. Marshall for his coöperation and interest in these experiments.

Immediately after the psychic disturbance, he showed (table 1) a rise in pulse, blood pressure, oxygen consumption, and cardiac output. By the time the second determination was made he had regained his composure and these functions are seen to have returned practically to their basal values.

In the case of subject H., the response to the psychic disturbance was a feeling of regret for his failings. This feeling increased subjectively with time and the physiological functions studied are observed to have also responded increasingly in the second determination.

Subject B. was little affected by the disturbance, considering the rebuke as directed, not towards himself, but rather at his associates. There was, in accord with the subjective psychic response, only a minimal reaction as recorded in the pulse, blood pressure, oxygen consumption, and cardiac output. The same applied to subject J.

In the first experiment quoted on subject C., an attempt to elicit a psychic disturbance was made by engaging the subject in heated conversation and detracting by various means the subject's attention from the experiment. Only slight changes were thus elicited.

Subject E. was aroused to extreme anger and showed a marked reaction. The first experiment quoted on subject G. was obtained indirectly. During the course of a series of determinations intended for another purpose, the subject was aroused to an extreme anger because of the carelessness of an assistant. The rise in cardiac output observed in an experiment performed immediately after this outburst of temper showed (as did also subject E.) the greatest rise in cardiac output (0.9 liter) which was elicited by psychic disturbances.

The last two experiments quoted, on subjects G. and C., show the absence of a response following slight disturbances (such as conversation, slamming a door, and the like). Such slight disturbances have produced no changes in the cardiac output of subjects, such as G. or C. who were trained in the technique of the experiment and accustomed to its performance. It is quite possible, however, that in the case of more neurotic subjects, or those unaccustomed to the procedure (*e.g.*, clinical cases) such slight disturbances might produce an appreciable effect on the cardiac output.

Discussion. The results of table 1 show definitely that psychic disturbances are capable of affecting not only the pulse and blood pressure, but may have an appreciable effect on the output of the heart. This increase in cardiac output is the accompaniment of a diminished arteriovenous oxygen difference. The slight increase in oxygen consumption noted after psychic disturbances is due probably to an increased muscular tone, rather than to any effect on the metabolic processes of the cells themselves.

In every case where a rise in cardiac output was observed as a result of psychic disturbance, there has been a concomitant change in the pulse and blood pressure. Conversely, in those cases where the pulse and blood pressure have not been affected, no change in cardiac output has been observed. The results do not, therefore, confirm the view of Collet and Liljestrand (1924) who claim that the cardiac output may be increased when the pulse and oxygen consumption have returned to normal. Moreover, the erratic results obtained by these investigators, on one of their subjects (M. E. C.) and which they attributed to emotional effects, are due, no doubt, to errors in their determinations as already noted (Grollman, 1928, 1929c).

The psychic disturbances utilized in the present investigation were all of a stimulating type. Certain emotional changes which are accompanied by a slowing of the pulse (Rihl, 1926) and a general decline in the activity of the circulation, may possibly result in a diminished cardiac output, while certain psychic reactions may possibly be without effect on the cardiac output.

The results presented in this paper emphasize the importance of avoiding all psychic disturbances in carrying out determinations on the cardiac output. The necessary precautions must be taken in order to obtain truly basal values. This is particularly true of subjects unaccustomed to the procedure. With reasonable care, however, normal resting values are easily obtained and the indiscriminate attribution of unexpected variations to psychic disturbances is unwarranted.

As regards the mechanism of the circulatory changes following psychic disturbances, the results indicate that there are other factors, besides vasomotor reactions, which affect the vascular system in emotional changes. Vasomotor effects alone might, by constriction in the splanchnic area and dilatation in the periphery, affect the blood pressure and blood flow through a given area as measured by the plethysmograph (Weinberg, 1923). The increased cardiac outputs observed indicate, however, that an acceleratory action on the heart itself also occurs. Whether this is brought about by the liberation of some substance into the blood stream, by impulses from the sympathetic or the vagus, by an increase in the amount of blood returning to the heart, or by a combination of these factors remains problematical.

SUMMARY

Psychic disturbances were found to increase the cardiac output from 0.1 (in the case of mild disturbances) to 0.9 (in the case of anger) liters. The pulse and blood pressure showed concomitant increases, while the oxygen consumption was only slightly increased. Mild disturbances, not accompanied by pulse or blood pressure changes, produced no change

in the cardiac output. The applications of these findings to the problem of the mechanism of the vascular changes accompanying psychic disturbances are discussed.

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BLOOD-FLOW THROUGH EDEMATOUS EXTREMITIES

An Experimental Study of the Effects on the Gaseous Content and the Volume-Flow of Blood of the Injection of Saline Solution

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The experimental studies which are described here were made as a supplement to some clinical observations which are to be reported elsewhere. Blalock (1926) determined the oxygen-content of blood from the femoral veins of patients who had unilateral varicose veins. It was found that the venous oxygen-content of the blood of the diseased side was higher than that of the normal side. Harrison and Pilcher (1929) determined the oxygen-content of blood from the femoral veins of patients with edema of cardiac origin. The effects of the partial disappearance of the edema on the oxygen-content were noted. It was found that the oxygen-content of the blood of the femoral vein was highest when the edema was most marked, and that the oxygen-content dropped as the edema disappeared. The high oxygen-content of the blood from the femoral vein, and hence the small arterio-venous difference in these two clinical conditions, may be due to one or both of two factors: 1, an increase in the blood-flow, or 2, a diminution of the oxygen-consumption. Since it was not possible to measure the actual flow of blood in the human, it was thought worthwhile to study the relationship between the arterio-venous difference in gaseous content and the volume-flow of blood in animals with unilateral edema. It is the purpose of this paper to report these studies.

Methods and results. Dogs were used in all of the experiments. The anesthetic employed in most instances was morphine. Saline solution was injected into the subcutaneous tissues and muscle of one leg until it was appreciably larger than the one which was to be used as a control. Hypertonic, isotonic, and hypotonic solutions of saline were used in different experiments. In several instances, the leg which was used as the control was stuck an equal number of times with a needle without the injection of any saline solution. When samples of blood were withdrawn for gas analyses, precautions against contact with air were observed. The blood gas analyses were performed on the Van Slyke-Neill manometric

apparatus. In the experiments in which the total flow of blood from the femoral veins was to be determined, cannulae were inserted quickly into the femoral veins and the total outflow measured.

I. *The effect on the oxygen and carbon dioxide content of the blood from the two femoral veins of the injection of saline solution into one of the posterior extremities.* In this series of observations, saline solution was injected into the subcutaneous and muscular tissues of the thigh and lower leg. The amount of saline solution which was injected in all experiments was 450 cc. This caused a definite visible enlargement in the size of the leg, which could also be detected by measurements.

Seven experiments of this type were performed. In one of these the oxygen-content of the venous blood before the injection of saline solution was considerably higher on one side than on the other and this experiment was therefore discarded. In the remaining six experiments, the results are all similar to two typical experiments which are recorded here. Both of these experiments were performed on one dog. In the first instance, isotonic saline solution was injected into the right leg, and nineteen days later the opposite leg was used.

Protocol: September 7, 1928, at 11:00 a.m. morphine 0.13 gram was injected into a male Airedale dog weighing 15.7 kilograms. At 1:50 p.m. control blood samples were obtained from the femoral veins. From 1:55 to 2:25 p.m. 450 cc. of isotonic saline were injected into the right posterior extremity. At 2:27 p.m., two minutes after the completion of the saline injection, blood samples were taken from both femoral veins. At 3:05 p.m., 40 minutes after saline injection, the third blood samples were withdrawn. At 4:10 p.m., 105 minutes after the saline was injected, the fourth blood samples from the two femoral veins were obtained and also a sample from one of the femoral arteries. During the experiment there was no decrease in the size of the leg into which saline had been injected that was detectable by vision. However, the next morning no enlargement was detectable.

The results obtained in this experiment are given in table 1.

Nineteen days later, the procedure was reversed, the right leg being used as the control and saline injected into the left leg.

Protocol: September 26, 1928 at 11:00 a.m., 0.13 gram morphine injected. At 2:10 p.m. control samples from the two femoral veins. From 2:15 to 2:45 p.m. 450 cc. of isotonic saline injected into the left leg. At 3:00 p.m. (15 minutes after the completion of saline injection) blood samples from the two femoral veins. At 3:40 p.m. (55 minutes after saline injection) blood samples. At 4:20 p.m. (95 minutes after saline injection) blood samples from both femoral veins and one femoral artery. No diminution in the size of the injected leg was noticeable at this time.

The results obtained in this experiment are given in table 2.

These two experiments, which are typical of the others which were performed, show that the oxygen-content of the blood of the femoral vein of the injected side is higher than is that of the control side. The oxygen-content of the arterial blood is the same on the two sides, and hence the difference in oxygen-content of the blood of the femoral artery and vein

is less on the injected side. The carbon dioxide content is lower in the leg into which saline was injected than in the control.

II. *The effect on the oxygen-content of the blood from femoral and popliteal veins of (a) unilateral injection of saline into the lower leg from the knee downward and (b) of extending the injection upward into the thigh.* Three experiments of this type were performed. Isotonic saline solution

TABLE 1

TIME	RIGHT FEMORAL VEIN (INJECTED SIDE)				LEFT FEMORAL VEIN (CONTROL SIDE)				FEMORAL ARTERY	
	O_2 content	A-V O_2 difference	CO_2 content	V-A CO_2 difference	O_2 content	A-V O_2 difference	CO_2 content	V-A CO_2	O_2 content	CO_2 content
	vols. per cent	*vols. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*
1:50 p.m. Control before saline injection	3.36	6.49	41.5		3.36	6.49	42.5			
2:27 p.m. 2 minutes after 450 cc. saline right leg	6.33	3.52	36.7		3.24	6.61	42.9			
3:05 p.m. 40 minutes after saline injection	5.16	4.69	37.6		3.70	6.13	43.5	7.3		
4:10 p.m. 105 minutes after saline injection	5.16	4.69	40.3	1.8	3.60	6.25	45.8	7.3	9.85	38.5

TABLE 2

TIME	RIGHT FEMORAL VEIN (CONTROL SIDE)		LEFT FEMORAL VEIN (INJECTED SIDE)		FEMORAL ARTERY	
	Oxygen content	A-V Oxygen difference	Oxygen content	A-V Oxygen difference	Oxygen content	Oxygen capacity
	vols. per cent	*vols. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*	
2:10 p.m. Control before saline injection	2.19	9.21	2.43	8.97		
3:00 p.m. 15 minutes after saline left leg	2.49	8.91	6.06	5.34		
3:40 p.m. 55 minutes after saline	2.85	8.55	5.46	5.94		
4:20 p.m. 95 minutes after saline	3.64	7.76	5.64	5.74	11.40	12.0

was used in each experiment. The popliteal veins were exposed at the level of the knee so that blood could be drawn from them without having to produce stasis. Blood samples were withdrawn simultaneously from the two popliteal veins as were those from the femoral veins. After control determinations had been made, isotonic saline solution varying in amounts from 200 to 300 cc. was injected into one leg below the knee. Injections were made into the subcutaneous and muscular tissues. After

varying intervals of time, samples of blood were drawn from the femoral and popliteal veins. The results of the analyses of the blood from the femoral veins were uniform in that the oxygen content on the side into which saline solution was injected was usually higher and never lower than that of the uninjected side. However, the oxygen content of the blood from the popliteal veins was variable. In one of the experiments, the content of the blood from the popliteal vein was always higher on the side into which saline solution had been injected, while in the remaining two experiments the contents varied, at times being higher on one side and later higher on the opposite side.

The results were of the same character when the injection was extended from the knee to the inguinal region. The oxygen content of the blood from the femoral vein was usually higher on the injected side while that of the blood from the popliteal veins was variable.

An experiment in which the oxygen content of the blood from the popliteal veins varied is described in detail.

Protocol: March 7, 1929, dog weight 12 kilograms. At 10:00 a.m. barbital 2.4 grams by stomach tube and morphine 0.1 gram by hypodermic injection. At 1:20 p.m. control samples of blood from both popliteal and both femoral veins. At 1:35 p.m. 200 cc. of saline solution injected into subcutaneous and muscular tissues between right knee and toes. At 1:55 p.m. (20 minutes after injection of saline solution) blood samples from popliteal and femoral veins. At 2:25 p.m. (50 minutes after injection of saline solution) blood samples from popliteal and femoral veins. At 2:35 p.m. 310 cc. of saline solution injected into right thigh. At 3:00 p.m. (25 minutes after injection of saline solution into thigh) blood samples from popliteal and femoral veins. At 3:25 p.m. (50 minutes after injection of saline solution in thigh) blood samples from popliteal and femoral veins.

The results obtained in this experiment are given in table 3.

III. *The effect on the outflow of blood from the two femoral veins of injecting saline solution into one leg.* This was determined by placing cannulae in the two femoral veins after saline solution had been injected into one leg and measuring the total outflow of blood from each vein. In some experiments, T-shaped cannulae were placed in the veins so that the blood could continue through the cannula to the heart except when the vein was clamped above the cannula and a measurement was being made. In other instances a straight glass cannula was used and the outflow was determined continuously. The amount of saline solution injected varied from 400 to 900 cc. Heparin was used as an anti-coagulant in one of the experiments. In another experiment, the legs were perfused with defibrinated blood after placing cannulae in the two femoral arteries. No anti-coagulant was used or perfusion done in the remaining instances.

Seven experiments of this type were performed. In one of these, the outflow was the same on the two sides. In the remaining six, the outflow was greater from the side into which saline solution had been injected,

the percentage increase over that of the control side varying from 50 to 400 per cent if the average figures of individual experiments are taken. Many single determinations showed a larger increase.

The protocol of a single, typical experiment will be given in detail.

Protocol: March 15, 1929. Weight of dog, 10 kilograms. At 12:00 noon, chloralose one gram intravenously. Four hundred eighty cubic centimeters of isotonic solution of saline were then injected into the leg from the inguinal region to the toes. At 1:40 p.m. heparin, 250 mgm. intravenously. T-shaped cannulae were then

TABLE 3

TIME	CONTROL SIDE		INJECTED SIDE	
	Femoral vein, oxygen content	Popliteal vein, oxygen content	Femoral vein, oxygen content	Popliteal vein, oxygen content
	vols. per cent	*vols. per cent*	*vols. per cent*	*vols. per cent*
1:20 p.m. Controls before saline	15.0	17.64	15.24	14.52
1:55 p.m. 20 minutes after saline (lower leg)	15.24	17.4	16.92	18.36
2:25 p.m. 50 minutes after saline (lower leg)	14.04	17.34	15.84	14.04
3:00 p.m. 25 minutes after saline (thigh)	15.36	17.7	17.04	18.36
3:25 p.m. 50 minutes after saline (thigh)	16.68	17.64	16.68	16.2

TABLE 4

TIME	CONTROL SIDE, FEMORAL VEIN		INJECTED SIDE, FEMORAL VEIN		FEMORAL ARTERY
	Flow per minute	Oxygen content	Flow per minute	Oxygen content	Oxygen content
	cc.	*vols. per cent*	*cc.*	*vols. per cent*	*vols. per cent*
2:20	15.0		69.0		
2:25	18.0	5.76	75.0	7.8	13.68
2:36	12.0	3.12	43.5	5.28	
2:47	10.5		37.5		
2:55	15.0		26.0		
3:50	27.0		36.0		

placed in the two femoral veins. At 2:20 p.m. femoral veins clamped proximal to cannula and measurements made of the blood flowing from both sides. At 2:25 and 2:36 p.m. measurements of flow and collection of samples for oxygen analyses. At 2:47, 2:55, and 3:50 p.m. measurements of flow.

The results obtained in this experiment are given in table 4.

IV. *The effect on the blood-flow of placing tourniquets around each leg in the inguinal region which occluded everything except the femoral artery and vein.* Because of the possibility that the injection of saline solution into an extremity might cause blood which ordinarily returns through other

veins to return through the femoral vein, tourniquets were placed around the legs but beneath the femoral artery and vein. This was done in two experiments. The flow of blood was greater in the extremity into which saline solution had been injected.

Discussion. These experiments show that the amount of blood which flows from the femoral vein is increased when saline solution is injected into the part. As was stated previously, these experiments were carried out in any effort to simulate true edema. The exactitude of this similarity is not known. It may be said that any purely mechanical effect of local swelling alone (e.g., of interfascial "pockets" of fluid) would be to diminish the blood-flow rather than increase it. Furthermore, as has been stated, the effect here is the same as that found as a result of true edema in patients,—at least, so far as gaseous content is concerned. It is also reasonable to assume that, by the time our final studies in each experiment were made, the diffusion of fluid in the tissues had resulted in a fairly uniform distribution. There is no reason to assume a specific chemical effect of true edema-fluid and for this reason it seems logical to conclude that the low utilization which had been found in patients with edema is primarily due to an increased minute blood-flow through the edematous extremities.

The increase in outflow from the leg into which salt solution was injected cannot be accounted for by dilution of the blood. The results of analyses upon samples of blood from the two sides showed that the oxygen capacity was no lower on the injected than on the normal side.

It is interesting to speculate as to the cause of the increase in outflow of blood from the femoral vein of a leg into which saline solution has been injected. In edema of the lungs it is known that there is a greater difference than normal between the oxygen tension of the alveolar air on the one hand and the blood on the other. Edema in any location probably produces anoxemia by imposing a barrier to the passage of oxygen. The effect of anoxemia on the circulation rate has been studied by many observers. Krogh (1924) found that it increased the coronary flow. Harrison and Blalock (1927) demonstrated an increase in cardiac output during anoxemia.

It seems reasonable to assume then that peripheral edema, like edema in the lungs, acts as a barrier to the passage of oxygen,—in this case, from the blood into the tissues. The compensatory mechanism is an increase in volume flow, since this maintains a higher oxygen tension in the capillaries. In the presence of long-standing, extensive edema, the increase in blood-flow, no matter how efficient the circulatory mechanism of the body, may fail eventually to compensate for the tissue anoxemia. (When the circulation is inefficient, as in heart disease, the failure in compensation must come all the sooner.) The significance of these changes is discussed in detail in preceding and subsequent publications.

It can be stated with certainty that the blood flow through edematous extremities is usually increased, but no positive statements can be made as to the exact location of the increase. It appears obvious that the gross mechanical effect of edema would act toward diminishing blood-flow, but in the preceding paragraph we have stated reasons for believing that the observed increase in blood-flow may be due to an impairment of oxygen diffusion. It is not illogical to think that the one effect may predominate in the less active tissues, and the other effect in the more active portions which have a greater need for oxygen.

SUMMARY

1. The oxygen and carbon dioxide content of blood from the femoral veins of both hind legs of dogs has been studied before and after the injection of saline solution into one leg, in attempted simulation of clinical edema, and the volume outflow from both femoral veins has been measured after such injection.

2. The oxygen-content of the femoral blood has been found to be higher in the "edematous" leg.

3. The carbon dioxide content of blood from the femoral vein of the "edematous" leg has been found to be lower than that of blood from the femoral vein of the normal leg.

4. The volume outflow of blood from the femoral vein of the "edematous" leg has been found to be from 50 to 400 per cent higher than that from the femoral vein of the normal leg.

5. The evidence here presented, with that reported previously, is believed to show that the volume flow of blood is greater than normal through edematous extremities.

6. It is postulated that the increased flow results from resistance by edema to passage of oxygen into the tissues; that the effect of this, in addition to (and in spite of) increased blood-flow, is tissue anoxemia. However, no definite statements can be made as to the exact location of the augmented flow.

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THE INFLUENCE OF DIET IN THE DEVELOPMENT OF MYXEDEMA IN THYROIDECTOMIZED PIGS

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In our earlier investigations of the effects of thyroidectomy on pregnant swine and their offspring, it was noted that certain diets apparently were influential in the intensity of the symptoms of thyroid deficiency and myxedema. This suggested more fundamental experiments to determine the extent of these dietary influences, and to form a basis for the future in analyzing the influence of diet on the character of the symptoms of myxedema in swine. These experiments were conducted to study the effect of foods of different protein content on thyroidectomized young swine.

Ten duroc pigs, litter mates from two related litters, two months old were used. They were divided into two groups of five each. One group was given a low protein and high carbohydrate diet; the other was given a high protein, low carbohydrate diet. The first diet consisted of shelled corn and water only, and had a nutritive ratio of 1 part protein to 10.4 parts carbohydrate; the second diet was composed of corn, oats, flour-wheat middlings and old process linseed meal, and had a nutritive ratio of 1 part protein to 3.9 parts carbohydrate. The fat in the diet has been converted into its carbohydrate equivalent (Henry and Morrison).

LITERATURE. Many investigations have been conducted to study the effect of feeding thyroid derivatives and iodine-containing compounds to normal and thyroidectomized animals. Hewitt (1920), Herring (1917), Hoskins (1916), and Cameron and Carmichael (1920) (1921), using white rats and rabbits, observed that thyroid feeding causes a decrease in growth and a loss of fat in normal animals. Cameron and Carmichael noted that this effect was not obtained when sodium iodide was fed. Thyroxine, when fed by mouth, produced the same effect as thyroid derivatives. The influence of thyroidectomy and the feeding of thyroid derivatives to thyroidectomized animals has been studied by many observers, among others Smith, Greenwood and Foster, Livingston, and Caylor and Schlotthauer. The effects of thyroidectomy alone have been studied by Mossu, Munk, Liddell and Simpson, Basinger, Kunde and Carlson, Caylor and Schlotthauer. These authors have performed thyroidectomy on various

animals and produced symptoms of thyroid deficiency. However, no one thus far has given special attention to the possible effect of different diets in the development of symptoms following thyroidectomy.

METHODS OF EXPERIMENTS. To avoid complications from intestinal parasites, all of these pigs were given anthelmintic treatment before being placed under observation. The next day they were confined five each in two barron paddocks (groups 1 and 2). After being photographed and weighed, two pigs in each lot were anesthetized with ether and thyroidectomy was performed. The remaining three pigs in each lot served as controls. The pigs in group 1 received the low protein, high carbohydrate diet (shelled corn), and those in group 2 received the high protein, low carbohydrate diet (corn, oats, flour-wheat middlings and linseed meal). The shelled corn was fed dry in a trough. Water was added to the grain mixture and it was fed as a thick mash. The pigs in each group were permitted to have as much food and water as they would consume. Group

TABLE 1

Swine on low protein high carbohydrate diet

	GROUP 1				
	Thyroidectomized swine		Control swine		
	Animal B 7	Animal B 11	Animal B 8	Animal B 9	Animal B 10
Total weight gained (kgm.)*.....	2.72	0.9	10.0	15.9	20.0

* The average gain in weight of the thyroidectomized swine was 1.81 kgm. and for the unthyroidectomized animals was about 15.3 kgm.

1 consumed a daily average of 3.8 pounds of shelled corn each; group 2 consumed a daily average of 2 pounds each of the grain mixture. Both groups of pigs were observed daily in regard to physical appearance and mental behavior. Individual weights were obtained each week. From the necessity of the conditions under which the experiments were conducted they were terminated after sixty days.

RESULTS OF EXPERIMENTS. *Group 1 (low protein, high carbohydrate diet).* The three normal pigs in this group gained more weight than the thyroidectomized pigs. The gain was 10, 15.90, and 20 kgm. respectively, an average gain of 15.30 kgm. They were active and appeared thrifty and healthy. Their hair coats and skin were smooth, and the fat on their bodies was evenly distributed.

The two thyroidectomized pigs in this group gained 2.72 and 0.90 kgm. respectively, an average gain of only 1.81 kgm. They remained small; the hair became rough or curly and the skin was wrinkled. This made them look like fat runts; they did not develop the edema of myxedema. In activity they resembled their normal litter mates

Group 2 (high protein, low carbohydrate diet). The three normal control pigs in this group gained 4.54, 7.27 and 7.27 kgm. respectively, an average gain of 6.36 kgm. They grew in stature but did not fatten as did the normal pigs in group 1. Their hair coat was quite long, although their skin was smooth. They were mentally alert and active.

The two thyroidectomized pigs in this lot gained 6.36 and 8.18 kgm. respectively, an average of 7.27 kgm., or 0.91 kgm. more than the controls. They appeared fatter and were mentally less alert than the normal pigs. They had drooping ears, long hair and wrinkled skin. Their general picture suggested early myxedema (table 1).[1]

COMMENT. The results obtained in these experiments demonstrate that diet is a significant factor in the development of the symptoms of myxedema, in thyroidectomized pigs. The normal pigs in group 1 (on a low protein high carbohydrate, diet) apparently were able to utilize the carbohydrate to good advantage. They grew in stature and stored fat on their

TABLE 2

Swine on high protein low carbohydrate diet

	GROUP 2				
	Thyroidectomized swine		Control swine		
	Animal B 1	Animal B 5	Animal B 2	Animal B 3	Animal B 4
Total weight gained (kgm.)*	6.36	8.18	4.54	7.27	7.27

* The average gain of weight in the thyroidectomized animals was 7.27 kgm. and in the unthyroidectomized animals 6.36 kgm.

bodies. There was nothing in their appearance that would indicate dietary deficiency. The thyroidectomized pigs in this group, however, manifested certain marked changes. They apparently were unable to eat more than a maintenance quantity of their ration. They failed to grow in stature and gained only slightly in weight. Growth was almost completely arrested. The edema of myxedema did not develop; the pigs were active and alert at all times.

The normal pigs in group 2 (on a high protein, low carbohydrate diet) grew in stature, but did not gain as much in weight as their litter mates in group 1. They looked rangy or lean. Their muscular development was good but they had little fat on their bodies. It was noted also that their hair was somewhat longer. They were mentally alert and otherwise nor-

[1] Myxedema is a term used in clinical medicine to designate the first of the two clinical syndromes of thyroid deficiency to be recognized. It is characterized by a group of symptoms consisting, among other things, of a peculiar edema associated with a mental retardation and loss of acuity of perception and activity.

mal. The thyroidectomized pigs in this group grew in stature with their litter mate controls. They appeared edematous and had gained slightly more in weight. These pigs had long hair, drooping ears, and wrinkled skin, and although the typical puffy, fat appearance which was obtained in our earlier experiments (1926) did not develop; because of their mental attitude they presented a more typical picture of thyroid deficiency than the pigs in group 1.

These results agree with those obtained in our experiments with adult pregnant sows. The thyroidectomized sows that received the balanced ration containing sufficient protein developed typical myxedema and the sows that received a low protein high carbohydrate diet did not; they lost weight, allowance being made for the weight of the uterine contents.

Boothby and his associates have shown in patients with myxedema that the edema of myxedema contains approximately 2 per cent nitrogen, and furthermore that the edema of myxedema seems to be due to the presence of an abnormal amount of deposit protein.

It is likely in our own experiments that the failure of the edema of myxedema to develop in the thyroidectomized swine on a low protein high carbohydrate diet was due to the absence of sufficient protein in their diets to provide for an abnormal amount of deposit protein, while on the other hand, the edema of myxedema was present in the high protein low carbohydrate diet group due to the presence of sufficient protein in the diet for the formation of an abnormal amount of deposit protein.

As has been noted, the thyroidectomized swine, receiving a low protein high carbohydrate diet, although obviously deficient in thyroid, did not develop myxedema and remained as alert as the normal controls. In contrast the thyroidectomized swine receiving a high protein low carbohydrate diet developed the edema of myxedema and became stupid, inert and lost their natural curiosity, so characteristic of young swine. This suggests that the mental symptoms, slowness and retardation, seen in swine and in patients with myxedema may be due in part to the pressure of the edema in the central nervous system.

SUMMARY

These experiments indicate that the quantity of protein in the diet is of significance in the development of edema of myxedema in thyroidectomized swine. Thyroidectomized swine on low protein high carbohydrate diet gained only slightly in stature and weight. Their normal litter mate controls, on the contrary, did not show ill effects from the diet, as was manifested by their growth and gain in weight.

The thyroidectomized swine on a high protein low carbohydrate diet grew in stature equal to their litter mate controls. However, they made greater gains in weight and developed myxedema. Their gain in weight probably was due to the edema so characteristic in thyroid deficiency.

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THE EFFECT OF THYROIDECTOMY AND OF CERTAIN DIETS ON PREGNANT SWINE AND THEIR OFFSPRING

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We have shown in a previous report (1926–1927) that swine are excellent animals for experiments on the thyroid gland, particularly those dealing with thyroid deficiency. In swine the thyroid gland has but a single lobe situated in the median line of the neck near the thoracic aperture, and it is supplied by a single artery. Because of its simple blood supply, its accessibility at operation, and its freedom of parathyroid bodies, thyroidectomy is easily performed. Swine are very susceptible to thyroid deficiency so that from ten to thirty days after thyroidectomy thyroprivia is apparent.

Our experiments were conducted to investigate the influence of certain diets on the development of myxedema[1] in thyroidectomized pregnant swine. The offspring were likewise studied at birth with reference to uniformity in size, viability, hair coat and size of the thyroid gland.

Twenty duroc sows aged one year were used in the experiments. They were divided into two groups and fed a balanced ration, and a ration low in protein and high in carbohydrates. The balanced ration was composed of corn, oats, wheat middlings, and oil meal; it had a nutritive ratio of 1 part of protein to 5.2 parts of carbohydrate (Henry and Morrison, 1917). The fat in the diets has been converted to its carbohydrate equivalent according to Henry and Morrison. The relatively low protein and high carbohydrate ration consisted of shelled corn and grass; it had a nutritive ratio of approximately 1 part of protein to 10 parts of carbohyrate. Twelve of the sows received 2 grains each daily of thyroid extract for varying periods.

LITERATURE. Many investigations have been conducted to study the effect of feeding thryoid and iodine-containing compounds to normal animals. However, white rats and rabbits were used in most of these experiments.

Hewitt (1914), (1918) noted that feeding large doses of thyroid gland

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to the normal white rat caused a decrease of growth, loss of fat and an increase in size of certain organs. Like results were obtained by Hoskins (1916), Herring (1917), (1920) Cameron and Sedziak (1921) and Cameron and Carmichael (1920–1921) (1921). The last authors, using white rats and rabbits, learned that feeding them thyroid caused a decrease in growth. This effect was not noted when sodium iodide was fed. Thyroxin when fed by mouth produced the same effect as thyroid. The effect of thyroidectomy and the feeding of thyroid and iodine-containing compounds on thyroidectomized animals has been studied by Smith, Greenwood and Foster, Marine, and Livingston. Smith, Greenwood and Foster injected a saline extract of fresh sheep thyroid glands into normal, thyroidectomized, and hypophysectomized rats. The normal rats apparently were not affected. The thyroidectomized rats receiving thyroid extract responded markedly. Their growth, and the weight of the suprarenal glands greatly exceeded those of their untreated thyroidectomized litter mates. Thyroid extract did not affect growth and did not cause restoration of the atrophied suprarenal glands, thyroid glands, and genital systems of hypophysectomized rats. Pituitary transplants caused a marked response. Marine states that the administration of iodine will prevent glandular hyperplasia in the remaining lobe of a partially thyroidectomized dog. Hammett (1926a, b, 1927) has made extensive studies of thyroidectomized white rats. His results were generally comparable to those observed by other investigators. Halsted found that extirpation of both lobes of the thyroid gland in dogs was fatal in from two to three weeks. Removal of one lobe caused early hypertrophy of the remaining lobe. Partial thyroidectomy of pregnant dogs caused hypertrophy and hyperplasia of the thyroid gland of the pups in utero. Livingston gave thyroid to normal and thyroidectomized rabbits of both sexes. Thyroidectomy caused pituitary enlargment in the male rabbit when thyroid was not given. Neither the normal nor thyroidectomized females were affected. Evvard and Culbertson demonstrated the great advantage that may result from the feeding of iodine to swine, as shown by the rate of growth and economy of gain. Hart and Steenbock ascribed the condition of hairless pigs to iodine deficiency. Kalkus, in a study of endemic goiter of newborn domesticated animals, stated that a sufficient quantity of iodine will prevent its occurrence. Smith, in his investigations in regard to iodine requirements of pregnant sows, found that daily doses of 5 grains of potassium iodide fed during the last four to five weeks of pregnancy would prevent goiter in newborn pigs.

METHOD OF EXPERIMENTS. *Series 1.* Experiments in this series were conducted during the summer of 1927. Ten sows were fed the balanced ration. They were one year of age and were all obtained from the same farm and bred to the same male. Immediately after breeding they were confined two each in six grass paddocks, numbered groups 1 to 6. Ten to

fourteen days later the sows in groups 1 to 5 were anesthetized with ether and thyroidectomy was performed. The two sows in group 6 were allowed to complete their gestation periods as unoperated controls. The animals in groups 1 to 4 received 2 grains each of desiccated thyroid for 30, 60, 90, and 120 days following thyroidectomy. Those in group 5 were used as thyroidectomized controls.

Series 2. Experiments were conducted during the summer of 1928. There were eight sows in this series. They were given a diet high in carbohydrates, consisting of shelled corn and green grass. All of the sows were obtained from one farm and were bred to the same male as those in series 1. Immediately after being bred they were confined two each in four barren paddocks, numbered groups 7 to 10. Ten to fourteen days later the sows in groups 7 and 8 were anesthetized with ether and thyroidectomy was performed. Each of two sows operated on in group 7 and the two normal sows in group 9 received 2 grains daily of thyroid extract. The two sows in group 8 were used as thyroidectomized controls and those in group 10 as normal controls.

Both series were observed with reference to physical appearance, attitude and individual weights. These observations were likewise made on the offspring at the time of farrowing. Complications due to intestinal parasites were avoided by anthelmintic treatment given as a routine. The experiments terminated at the time of farrowing.

RESULTS IN SERIES 1 (TEN SOWS FED A BALANCED RATION). *Group 1.* These sows were thyroidectomized and 2 grains desiccated thyroid extract were given for thirty days; typical edema of myxedema did not develop. The only symptoms noted were that they stopped eating the available green food and became quite inactive about three weeks after the thyroid extract was discontinued. The sows in this lot farrowed fifteen pigs, fourteen of which were alive. They were normal in regard to size and hair coat. None had gross enlargement of the thyroid gland.

Group 2. These sows were thyroidectomized and 2 grains desiccated thyroid extract were given for sixty days. The animals exhibited the same symptoms as those in group 1, but they were less marked. One sow farrowed six live pigs and one stillborn pig. The other sow was unable to deliver her offspring; two dead pigs were removed by caesarean operation. The mother recovered uneventfully. The pigs in both litters were normal in regard to size and hair coat. Thyroid enlargement was not visible.

Group 3. These sows were thyroidectomized and given 2 grains desiccated thyroid extract for ninety days. The animals were physically indistinguishable from the normal controls in group 6. One of these sows was not pregnant. The other farrowed a litter of normal pigs.

Group 4. These sows were thyroidectomized and 2 grains desiccated thyroid extract was given to term. The animals grew in height but

failed to fatten. They were the most active swine in the entire series. They ate all of the available green food in their paddock and even skinned the bark off of the trees. They ate the food given them greedily, fighting each other away from the trough. There was nothing about them suggestive of myxedema; on the contrary they were evidently hyperthyroid. These sows farrowed thirteen live pigs, in litters of six and seven. Twelve were of large uniform size and one was small but strong. Both litters were normal in regard to hair coat and the size of the thyroid gland.

Group 5. These sows were thyroidectomized controls. They were very quiet. They grew fat and approached the condition which we have previously described as suggestive of myxedema, namely, thickening of the neck and legs, wrinkled skin and coarse excelsior-like hair (Cameron and Carmichael, 1921). They ate almost none of the available green food in their paddock. During the last hundred days of their gestation period, they consumed less food and drank less water than any of the other sows. The sows in this lot farrowed seventeen pigs, in litters of seven and ten. Fourteen were alive. The litter of seven included one small stillborn pig and six large uniform sized pigs. The pigs in the other litter were of irregular size, and two were stillborn. The pigs of both litters had normal hair coats; the thyroid gland was not enlarged.

Group 6. These sows were controls and apparently normal in every respect. They farrowed eighteen pigs, nine each. The pigs in both litters were uniform in size. There were no stillbirths, although there were several weaklings. All were normal in regard to hair coat and size of the thyroid gland. Changes in weight and results of farrowing may be noted in table 1.

RESULTS IN SERIES 2 (EIGHT SOWS FED RELATIVELY LOW PROTEIN AND HIGH CARBOHYDRATE DIET). *Group 7.* These sows were thyroidectomized and 2 grains desiccated thyroid extract were given to term. The sows consumed about the same quantity of corn and grass as the control sows in group 10. They grew and ate like the normal animals in group 9. They were not so active as the sows in group 4. One sow farrowed seven live pigs at term. The other farrowed, ten days prematurely, twelve stillborn pigs. However, both litters were normal in regard to hair coat; the thyroid gland was not grossly enlarged.

Group 8. The sows in this lot were thyroidectomized controls. They presented an entirely different picture than those in group 5, series 1. They remained active and alert and consumed about the same quantity of shelled corn and green food as the sows in group 7. They did not refuse to eat green food at any time in their gestation period. One of the sows gained 7.72 kgm. and the other 9.5 kgm.; however, neither one was fat or myxedematous. They appeared thin, and to have lost flesh. One sow farrowed five live pigs at term; the other farrowed eight stillborn pigs ten

days prematurely. Both litters were normal in regard to hair coat. Thyroid enlargement was not visible. Indeed, in one pig the gland was not definitely demonstrable. Two very small accessory thyroid glands were all that could be found.

Group 9. These sows were normal unthyroidectomized swine and were given thyroid extract to term. They appeared active and alert. They consumed a regular quantity of corn and grass, appeared normal, and grew fat. One of these sows farrowed three live pigs and one stillborn pig; the other farrowed ten stillborn pigs. Both litters were at term. They were normal in regard to hair coat and gross enlargement of the thyroid gland was not observed. There was no evidence of hyperthyroidism as seen in series 1, group 4.

Group 10. These sows were unoperated controls and were given corn and grass and were not given thyroid extract. They appeared normal and ate well. They were physically indistinguishable from the sows in group 9, although the swine in group 9 made greater gains in weight. Both of these sows farrowed stillborn litters of pigs; one thirteen pigs at term, the other six pigs fourteen days prematurely. The pigs were normal in regard to their hair coat, and thyroid enlargement was not visible. Changes in weight and results of farrowing may be noted in table 2.

HISTOLOGY.[2] The thyroid glands of all pigs in which the gland was removed at birth were much alike, that is, of the fetal type. The cellular elements predominate over the colloid. Such colloid as was present was confined in very small acini. Most of the lining cells were cuboidal to columnar.

COMMENT. The symptoms of thyroid deficiency apparently were influenced by the kind of food ingested. It was noted that the thyroidectomized control swine in group 5 that were given a balanced diet developed symptoms of myxedema, while the thyroidectomized control sows in group 8, that were given a relatively low protein, high carbohydrate diet, did not develop the commonly recognizable symptoms of myxedema, but lost flesh. Their small gain in weight was not sufficient to compensate for the increase in weight of the uterine contents. Symptoms of thyroid deficiency did not develop in the thyroidectomized sows of either series when sufficient thyroid extract was administered for sixty days or longer.

The kind of diet employed seems to have an influence on the body changes in thyroid deficiency. Thyroidectomized animals on a relatively low protein high carbohydrate diet although obviously thyroid deficient did not develop the edema of myxedema. Boothby and his associates have shown in their observations on persons with myxedema that the edema of myxedema seems to be due to the presence of an abnormal

[2] The histologic changes found in these glands will be described in more detail in a subsequent article.

TABLE 1

Series 1. Thyroidectomized sows fed balanced ration

	THYROID EXTRACT								THYROIDECTOMIZED CONTROLS		NORMAL CONTROLS	
	Group 1, for 30 days		Group 2, for 60 days		Group 3, for 90 days		Group 4, for 120 days		Group 5, no thyroid extract		GROUP 6	
	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Animal 6	Animal 7	Animal 8	Animal 9	Animal 10	Animal 11	Animal 12
Weight gained, kgm.	56.36	54.09	60.9	50.0	42.27	40.0	30.45	40.45	48.11	35.45	44.09	31.81
Pigs in litter	6	9	7	2	6	None	7	6	7	10	9	9
Condition of pigs*	All alive	1 still-born; 8 alive	1 still-born; 6 alive	All still-born (caesarean)	Alive		Alive	Alive	3 still-born; 4 alive	Alive	Alive	Alive
Average weight at birth, grams	1560	1450	1400	1080	1560		1410	1800	1320	1080	1320	1080
Average weight of thyroid gland at birth, gram	0.20	0.22	0.3	0.02	0.23		0.12	0.25	0.7	0.24	0.34	0.39

* The hair on all of the pigs was normal at birth.

TABLE 2

Series 2. Sows fed corn and green food

	SOWS OPERATED ON				SOWS NOT OPERATED ON			
	Thyroid extract given		Thyroid extract not given		Thyroid extract given		Thyroid extract not given	
	Group 7		Group 8		Group 9		Group 10	
	Animal 13	Animal 14	Animal 15	Animal 16	Animal 17	Animal 18	Animal 19	Animal 20
Weight gained, kgm.	12.72	21.36	7.72	9.54	34.09	29.54	17.7	24.09
Pigs in litter	7	12	8	5	10	4	13	6
Condition of pigs*	Alive	Dead; ten days premature	Dead; ten days premature	Alive	Dead	1 stillborn; 3 alive	Dead	Dead; fourteen days premature
Average weight at birth, grams	807	648	768	1560	922	1400	792	796
Average weight of thyroid gland at birth, gram†	0.17	0.13	0.18	0.25	0.15	0.27	0.14	0.12

* The hair on all of the pigs was normal at birth.

† The largest thyroid glands were noted in the largest pigs.

amount of deposit protein. These animals in our experiments probably did not develop the edema of myxedema because of a lack of sufficient protein.

The thyroidectomized control sows in group 5, receiving a balanced diet, did not eat green food with the same zest as the thyroidectomized sows in this series that received thyroid extract. The craving for the green food seemed to be influenced by the quantity of thyroid extract ingested. This peculiar dietary phenomenon was not observed in the thyroidectomized sows in series 2, receiving the food high in carbohydrates. However, the two series are not wholly comparable, for the animals in series 2 were fed greens in a trough, while those in series 1 were forced to hunt for their green food.

Throughout the so-called goiter areas of North America, thyroid enlargement in the newborn is frequently seen in farm flocks. This is attributed to deficiency of iodine in the food. In swine the most manifest symptoms of this condition are hairless pigs. The pigs are generally carried to full term and often four to seven days overtime. If born alive, they usually die within a few hours. Their necks appear thick and pulpy; this enlargement, according to Hart and Steenbock, is due to the increased size of the thyroid gland. The disease, as manifested by the symptoms described, was not observed in any of the pigs in these experiments. Even the thyroidectomized control sows in series 2, that received shelled corn and grass only, farrowed pigs with normal hair coats and normal thyroid glands. However, the development of goiter and hairless pigs may be associated with iodine deficiency. In these experiments only thyroid deficiency was produced and this is a very different thing from iodine deficiency and produces different results.

SUMMARY

These experiments indicate that thyroidectomy does not affect pregnant sows if they receive 2 grains each of desiccated thyroid extract for sixty days or more following thyroidectomy. The symptoms of thyroid deficiency in pregnant sows were influenced by the diet of the animals. Thyroidectomized animals on a balanced diet revealed signs of myxedema whereas those on a high carbohydrate, low protein diet did not develop the edema of myxedema and were underweight (allowance being made for pregnancy). The hair coat and thyroid gland of the offspring of thyroidectomized pregnant swine were not markedly different from the offspring of the control animals. The thyroid gland was not enlarged in offspring of thyroidectomized swine, although in each group there was considerable variation in the size of the glands as indicated by the weights (table 1).

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THE EFFECT OF BILATERAL OVARIECTOMY IN CATS UPON SENSITIVITY TO INSULIN

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The object of these investigations was to determine the effect of ovariectomy, in cats, upon the sugar metabolism as evidenced by the sensitivity to insulin before and after the removal of both ovaries. Although the effect of ovariectomy on metabolism has been the subject of numerous investigations, there seems to be no reference in the literature where this approach has been utilized. As early as 1899 Loewy and Richter found that the removal of the ovaries caused a decrease in the consumption of oxygen in experimental animals. Since then a mass of literature has accumulated relative to the effect of ovariectomy on the basal metabolism both in animals and in humans. Aub and Taylor (1922) in a review of the literature felt that there was some indication that gonadectomy in animals causes a slow fall in metabolism (as much as 15 per cent) in three weeks. It seemed as if the change might be due to the disturbance of the normal interaction between the sex glands and the thyroid. Rowe and Lawrence (1926) felt that the evidence at the time was contradictory and opinion was far from unanimous. They were of the opinion, however, that they had shown a "slight but unmistakable depression of the respiratory metabolism" following ovariectomy. Plaut and Timm (1924) reported a fall, manifesting itself with the onset of the amenorrhea, of 100 to 200 calories in twenty-four hours following x-ray castration in women. Women past the menopause gave no such response. After six months the women who had previously shown a fall in metabolism showed a return to the original values in the five cases reported.

METHOD. Healthy, vigorous, female cats were selected for the experiment. Such animals have been found to give rather uniform results following the administration of insulin when used over a long period of time. They were fed on a diet consisting of bread, milk, and meat. Before being used in an experiment the cats were fasted for a period of twenty-four hours. The peripheral ear vein was used for obtaining blood samples, and the blood sugar content was determined by the Folin (1923) method. With a small syringe armed with a small needle the insulin (Squibbs-1 cc. = 20 units) was injected under the skin of the abdomen. Observations upon the

response of the intact animal toward insulin were made prior to operation; the dosage sufficient to produce convulsions within three hours was approximated. Following operation, from which recovery was uneventful, the response to insulin was determined at intervals.

In recording the response to insulin a fasting blood sugar determination was made; the blood sugar level was again determined one hour and thirty minutes following the injection, and again at the end of a three-hour period unless convulsions were produced, whereupon a blood sugar reading was made within five minutes thereafter. Besides noting the effect of the insulin upon the blood sugar level, the general reactions were observed and interpreted as slight, moderate, severe, and convulsive (see Britton, Geiling, and Calvery, 1928).

Under ether anesthesia through a lower-abdominal midline incision both ovaries were removed with the maintenance of aseptic precautions and with as little trauma as possible to surrounding tissues. At operation one cat showed a virginal uterus, one an early pregnancy and one a moderately-advanced pregnancy. The remaining four cats showed non-pregnant parous uteri.

The determinations of the responses to insulin were made four or five days after operation; these were repeated at intervals at no time shorter than five days over a period of a month and again at longer intervals. Four cats were observed for a period of between seven and a half and eight months. The remaining three animals died during the course of the experiment.

Results. The results of the experiments on four cats observed over the whole period are summarized in table 1.

Before ovariectomy cat 62 showed a slight reaction three hours after injecting five units of insulin per kilo body weight and convulsions were observed in one hour and forty minutes following the injection when the dosage was increased to six units per kilo body weight. Four days after operation two and seventy-five hundredths units per kilo body weight produced convulsions in two hours and five minutes from a fasting blood sugar level somewhat higher than the blood sugar level before operation. The response was practically the same eleven days later. One month after operation two and seventy-five hundredths units per kilo body weight produced only a severe reaction with the lowest blood sugar level determined at 55 mgm. per 100 cc. It is noteworthy that the blood sugar was increased at the three hour sample over that of the one and a half hour sample. Eight days later, however, a dosage of three units per kilo body weight produced convulsions in two hours and twenty minutes with a corresponding lowering of the blood sugar. A change was again noted three and a half weeks later when three units per kilo body weight gave only a moderate reaction with a correspondingly moderate depression of

TABLE 1

Experiments showing the responses of cats to the administration of insulin before and after bilateral ovariectomy

CAT NUMBER	DATE	WEIGHT	INSULIN GIVEN (UNITS PER KILO)	INITIAL (FASTING) BLOOD SUGAR	BLOOD SUGAR 1½ HOURS AFTER INSULIN	THIRD BLOOD SUGAR READING	TIME THIRD BLOOD SUGAR		GENERAL REACTIONS
		kgm.					*hrs.*	*minutes*	
62	4/10/28	1.92	4.00	70	42	47.5	3	00	Absent
	4/16/28	1.75	5.00	88	47	33	3	00	Slight
	4/20/28	1.75	6.00	72	32				Convulsions (1 hr. 40 min.)
	4/23/28	1.65	(Bilateral ovariectomy)						
	4/27/28	1.59	2.75	97	46	60*	2	10	Convulsions (2 hrs. 5 min.)
	5/ 3/28	1.53	2.75	112	30	43*	1	45	Convulsions (1 hr. 35 min.)
	5/ 8/28	1.45	2.75	77	34	41*	2	05	Convulsions (2 hrs.)
	5/22/28	1.62	2.75	100	55	65	3	00	Severe
	5/30/28	1.55	3.00	75	35	26*	2	25	Convulsions (2 hrs. 20 min.)
	6/22/28	1.85	3.00	78	55	47	3	00	Moderate
	10/ 1/28	2.23	6.00	66	50	45*	2	45	Convulsions (2 hrs. 40 min.)
	12/12/28	2.37	6.00	70	55	50	3	00	Slight
63	4/10/28	2.32	4.00	110	45	37.5	3	00	Absent
	4/16/28	2.44	5.00	102	37	25.5*	3	00	Convulsions (3 hrs.)
	4/20/28	2.55	4.50	110	52	40	3	00	Moderate
	4/23/28	2.53	(Mod. advanced pregnancy) (Bilateral ovariectomy)						
	4/25/28		(Pregnancy aborted)						
	4/27/28	2.27	2.25	110	55	46.5*	2	20	Convulsions (2 hrs. 15 min.)
	5/ 3/28	2.20	2.25	102	50	44*	3	00	Convulsions (2 hrs. 55 min.)
	5/ 8/28	2.22	2.50	81	44	37*	2	25	Convulsions (2 hrs. 20 min.)
	5/22/28	2.17	2.50	105	55	60	3	00	Slight
	5/30/28	2.31	3.00	82	36	35	3	00	Convulsions (3 hrs.)
	6/22/28	2.50	3.00	72	39	37	3	00	Slight
	10/ 1/28	2.75	5.00	74	50	42	3	00	Slight
	12/12/28	3.10	6.00	75	50	45	3	00	Absent
64	4/ 4/28	2.72	4.00	98	54.5	45	3	00	Absent
	4/11/28	2.80	5.00	70	35	31	3	00	Absent
	4/17/28	2.72	6.00	86	50	42	3	00	Severe

TABLE 1—*Concluded*

CAT NUMBER	DATE	WEIGHT	INSULIN GIVEN (UNITS PER KILO)	INITIAL (FASTING) BLOOD SUGAR	BLOOD SUGAR 1½ HOURS AFTER INSULIN	THIRD BLOOD SUGAR READING	TIME THIRD BLOOD SUGAR		GENERAL REACTIONS
		kgm.					*hrs.*	*minutes*	
64	4/23/28	2.53	7.00	82	51	44	3	00	Severe Convulsions (3 hrs. 15 min.)
	4/30/28	2.47					(Bilateral ovariectomy)		
	5/ 4/28	2.40	3.50	110	77	45	3	00	Moderate
	5/11/28	2.49	4.00	90	52.5	55	3	00	Severe
	5/22/28	2.36	4.50	108	53	48	3	00	Severe
	5/30/28	2.52	5.00	86	41	35	3	00	Severe
	6/22/28	2.80	5.50	78	45	43	3	00	Slight
	10/ 1/28	3.15	7.00	80	45	50	3	00	Slight
	12/12/28	3.25	8.00	80	46	45	3	00	Absent
96	4/19/28	2.42	4.00	90.5	55	45.5	3	00	Absent
	4/25/28	2.33	5.00	93	52	38*	2	45	Convulsions (2 hrs. 40 min.)
	4/30/28	2.35					(Bilateral ovariectomy)		
	5/ 4/28	2.12	2.50	96	56	53	3	00	Severe
	5/11/28	2.23	3.00	88	55	67	3	00	Moderate
	5/30/28	2.27	3.50	85	46	50	3	00	Slight
	6/22/28	2.45	4.50	77	50	45	3	00	Absent
	10/ 1/28	2.79	5.00	77	45	60	3	00	Absent
	12/12/28	2.97	6.00	75	50	55	3	00	Absent

* Blood sugar sample collected during or after convulsions.

the blood sugar level. Almost six months after the operation six units per kilo body weight, i.e., twice the dosage which five months before gave convulsions in two hours and twenty minutes, produced convulsions at two hours and forty minutes. The same dosage a month and a half later brought about only a slight reaction.

Cats 63, 64, and 96 demonstrated a similar variation in sensitivity after operation in that a smaller dose of insulin produced marked reactions. A gradual recovery of resistance to insulin was likewise observed. The moderately advanced pregnancy in cat 63 had no apparent effect on the response to these relatively large doses of insulin. In cat 64 three and a half and four units per kilo body weight following operation produced more serious general effects than did five units before operation with, however, less effect upon the blood sugar level. With large doses six and seven and a ha'f months after operation there was little, if any, general reaction with a lowering of the blood sugar relatively marked as compared

with the reaction obtained with smaller doses previously used. Cat 96 showed a similar discrepancy between the blood sugar levels and the general reactions.

Two cats died during July. Both these animals, one of which showed at operation a virginal uterus, followed the foregoing animals in demonstrating a similarly increased sensitivity to insulin following ovariectomy. Both animals, furthermore, showed a tendency to recovery of resistance as the period of observation lengthened. One cat was found dead on November first. This cat demonstrated equally well not only the immediate increase in sensitivity to insulin, but also the subsequent recovery.

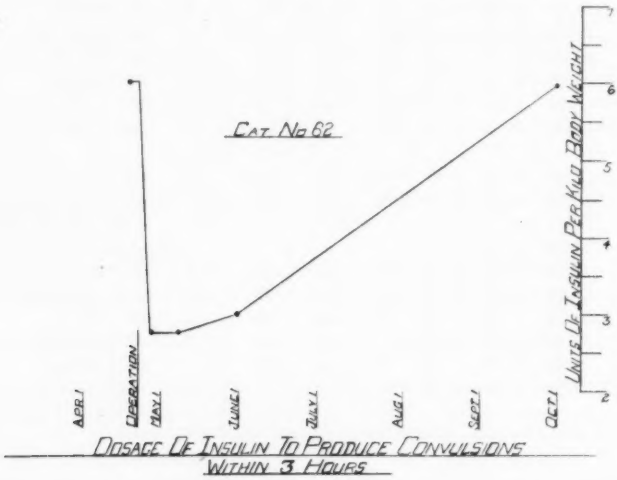

Chart I

Six months following operation the sensitivity to insulin was approximately that of the pre-operative state.

Bilateral ovariectomy in cats apparently decreases the immediate resistance of the organism to insulin so that a much smaller dose—approximately 50 per cent of that used before operation—will produce convulsions within about the same time as in the pre-operative condition. The resistance to rather large doses of insulin increases gradually. Seven and a half months after operation the dose of insulin sufficient to produce convulsions in three hours before operation has a decidedly milder effect. Indeed the resistance is such that even larger doses fail to produce reactions comparable to those observed before operation, i.e., in the intact animal. The blood sugar determinations demonstrate a similar, although somewhat less

pronounced variation in the effectiveness of insulin. Chart 1 shows graphically this alteration in the dosage of insulin per kilo body weight to produce convulsions within an arbitrarily determined period of time.

In cat 64 a blood sugar value of 45 mgm. per 100 cc. accompanied at the end of the three-hour period on the fourth of May a reaction interpreted as moderate and on the twelfth of December no apparent reaction. A value of 44 mgm. per 100 cc. accompanied a severe reaction on the twenty-third of April. On the eleventh, twenty-second, and thirtieth of May, with blood sugar levels of 55, 48, and 35 mgm. per 100 cc. respectively, the reaction in each case was interpreted as severe in character. Reference to the table will furnish additional occurrences of the same nature. That the general reaction and the blood sugar levels do not correspond would seem to be indicative that the so-called hypoglycemic convulsions may be of such a nature that the hypoglycemia and the convulsions do not exist

TABLE 2

Observations on the increase in weight in cats following bilateral ovariectomy

CAT NUMBER	WEIGHT		PERCENTAGE GAIN IN WEIGHT
	At operation	December 12	
	kgm.	*kgm.*	*per cent*
62	1.65	2.37	44.5
63	2.53*	3.10	22.5
64	2.47	3.25	30.8
96	2.35	2.97	26.4
Average			31.5

* Moderately advanced pregnancy aborted following operation.

in a cause and effect relationship but may be more or less independent manifestations of the effect of the insulin.

Discussion. The withdrawal of function of the ovary evidently upsets the preëxisting equilibrium in the endocrine system in the control of the metabolism of sugars. The presence of the ovaries certainly is of value in the organism's response to large doses of insulin in mobilizing the body's resources in combating the influence of the insulin. Following a preliminary depression of resistance to insulin there is a gradual recovery of the protective mechanism, if it may be called such, against the violence of insulin suggesting a realignment of values among the other glands of internal secretion. A similar disturbance and recovery is suggested by studies in the epinephrine sensitivity in women at the menopause (Myers and King). Furthermore it seems that following the loss of function of the ovaries and subsequent reëstablishment of balance among

the endocrine glands this reorganization may be such that there is no longer the same relationship of the response of the carbohydrate metabolism and the response of the central nervous system to large doses of insulin.

The four remaining cats were sacrificed and autopsied after the last determination of the response to insulin had been made. Nothing essential was found in any of the cats except that the thyroids and adrenals, excepting cat 62 where they appeared of normal size, seemed somewhat smaller than would be expected in cats of their size and weight. This corresponds with the observations of Hatai (1915) that following ovariectomy in white rats there is a decrease in the size of the adrenal bodies. No gross evidence of ovarian tissue was found in any of the cats.

With one exception and that where pregnancy was advancing, these cats had shown a slow gradual loss of weight from the beginning of the experiment coincident with their confinement to the animal quarters, and continuing a short time following operation. At the end of the period of observation, however, these cats (table 2) presented a marked gain in weight, amounting to an average of 31.5 per cent over their pre-operative weights. The change in the economy of these cats was sufficient not only to overcome the tendency toward loss of weight in this unnatural environment but also to show quite a marked absolute increase in weight following ovariectomy. The cat observed for just six months showed an exactly similar change in weight. In this cat there was an increase of 21.0 per cent over the pre-operative weight in a period of six months.

SUMMARY

Bilateral ovariectomy in these cats caused an immediate increase in sensitivity to insulin.

There was a gradual recovery of resistance to insulin to such an extent that the sensitivity was subsequently diminished in comparison with that of the intact animal.

Blood sugar levels and the general reaction to insulin did not coincide from time to time. This would suggest that insulin shock and hypoglycemia are separate and distinct manifestations resulting from the injection of insulin. There seems to be an exaggeration of their independence following the recovery of the animal from the immediate effects of ovariectomy.

Bilateral ovariectomy was followed in these cats by a marked and unmistakable increase in weight.

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THE REACTANCE OF NERVE AND THE EFFECT UPON IT OF ELECTRICAL CURRENTS

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In this paper are described certain effects upon nerve of injurious electrical currents.[1] On the supposition that the "capacity," that is, polarizability, of nerve is bound up with its functioning, the nerve has been studied physically as a reactive electrical circuit, in an attempt to find the locus in the nerve, and the value of the effective capacity in the normal state. The nerve was then injured by passing currents through it, and the change in reactance was correlated with alterations in the functioning of the nerve as measured by the action potential. Nerve was selected for this study, first, because of its relative simplicity as a conductor, second, because of the precision with which its response can be measured, and third, because of the fact that many concrete and quantitative data have been accumulated as to the properties of nerve. It is not intended to imply that the functioning of nerve is any simpler than that of other tissues; only that a greater number of apparently simple things are known about it. Nerve therefore serves as a type of irritable structures. The data obtained from nerve as to general mode of function and effects of injury are presumably applicable to other irritable tissues.

The "capacity" of nerve, in which factor is here included the polarizability of its membranes or interfaces, has generally been recognized as being bound up with its biological functioning. However, it is obvious, from the complex structure of the axon, that polarization may take place at different regions along the path traversed by electric currents flowing through the fiber. The same considerations apply to the nerve trunk as a whole, as usually investigated, where different axons may have different effective capacities, and where connective tissue surrounds the nervous elements. It is to be expected a priori therefore that any given nerve has many different "capacities," but heretofore no differentiation between them has been attempted.

If some one critical surface is involved in nerve activity, alteration of

[1] Published by the committee on physiology of the Conference on Electrical Shock.

this surface should result in a more or less parallel alteration of both irritability and intensity of response of the nerve. The *polarizability* of this particular surface should, other things being equal, be one index of its functional state. The polarizability of other interfaces might, however, be differently affected, or not at all, by agents which alter the nerve's functioning. Differentiation between the polarizability of different structures has not been satisfactorily demonstrated, though certain inferences may be drawn as to the spatial distribution of the capacities which are demonstrable. As an agent for altering the polarizability of such a membrane, high voltage induced currents have been employed, on the assumption first, that the polarizability of the membrane should be susceptible of significant alteration by an agent which is able to polarize it, and second, that alternate make and break shocks should produce "injury" with as little complication in the way of chemical or mechanical alteration as any other. Each induced shock, although alternating in direction, polarizes the nerve, and is effectively a "direct" current, in its injurious action, the nerve depolarizing between shocks. Heat and mechanical crushing were also employed as agents of injury.

A distinction may be emphasized here between polarization and polarizability. Nerve is polarized by its own resting activity, as shown by the possibility of obtaining the demarcation current. The interface so polarized is therefore polarizable. Other structures, however, are also polarizable, even though in uninjured nerve, they may be unpolarized. Depolarization may be looked upon as negative polarization. In general, then, all the polarizable structures in nerve may be polarized either by the nerve's action currents or by currents applied from the outside, though not all are initially polarized.

1. *Apparatus.* The apparatus involved consisted of a capacity bridge, an especially designed pendulum interrupter, a potentiometer for a supply of direct currents to the bridge, and an induction coil and capacities as a source of alternating currents for resistance measurements; the cathode ray oscillograph was employed as a null instrument for detecting a bridge imbalance, and also for recording the nerve polarization and action potentials as transient imbalances. Using a three-stage shield plate tube amplifier, the sensitivity of the whole was up to 150 meters deflection per volt output from the bridge. The bridge was made symmetrical, including the battery supply and leads to the interrupter, for these elements, in so far as they have capacity, are effectively in the bridge arms (fig. 1). Further details of the bridge and oscillograph arrangements employed are appended to this paper.

The nerve was mounted in a moist chamber on nonpolarizable electrodes, with fine yarn wicks (1 mm. wide usually). The mercury surface of each electrode was 5 sq. cm. and the electrolyte was Ringer's solution with an excess of calomel. Threads tied to either end and passing to adjustable glass rods supported the nerve in a slightly stretched position. As a source of injurious current, a Porter coil with iron core was connected to deliver faradic shocks using two dry cells in series. To one wire of a metal stimulating pair connected to this coil a yarn wick was attached. By

manipulating this stimulator from outside the chamber, the bare wire could be touched to the yarn wick of one calomel electrode, and the yarn leading from the other wire placed on the nerve at a distance, distal with respect to the other calomel electrode, without disturbing the nerve (fig. 1, *E*, *F*). The injury from the shocks thus involved the tissue under only one of the lead electrodes, and no current passed through the calomel electrode proper.

The electrodes were tested for polarizability by connecting their yarn wicks together so that the resistance was about 10,000 ohms (10 mm. yarn) and the bridge balance recorded with both alternating and direct current. To alternating currents no capacity could be detected at frequencies of 300 to 2000 per second, but to direct currents a very slight shift of voltage occurred, approximately 0.002 millivolt per

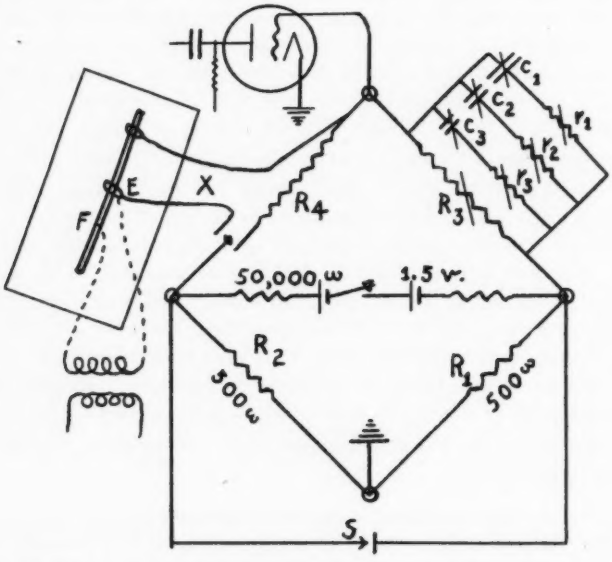

Fig. 1. Wheatstone bridge diagram for measurement of polarizability of nerve by use of galvanic currents of short duration. Details in text.

0.001 second, under an applied voltage across the bridge of 37½ millivolts. Since this electrode resistance is only about one-sixth that of one centimeter of nerve trunk, and one-tenth that of the nerve root, the currents passing through the electrodes during an experiment were correspondingly less, and this polarization, while it might be detectable under the conditions of the experiment, proves to be small compared to the similar polarization component of the nerve.

2. *The character of the capacity of nerves.* Certain theoretical assumptions being convenient in the analysis of a practical problem such as that of tissue injury, the following hypothesis is employed as to the essential nature of nerve capacity. In the first place, the data given are in terms of

effective capacity, that is, a length of nerve in contact with two electrodes, considered as an electrolytic conductor with reactance, *has the same effect as* a certain capacity-resistance network, upon the currents which pass through it. Secondly, the capacity is inferred to be largely polarization capacity, essentially similar to an electrolytic condenser or a capillary electrometer. In parallel, and more or less insulated, conductors there should be some true capacity and inductance, with the insulation as dielectric, in the Maxwellian sense (Goethlin, Grehore and Williams, etc.). This should, however, be relatively small. Such a capacity would correspond to that of two conducting plates closely approximated, but separated by a dielectric. Now if one of those plates is lead and the other aluminum, and if the "dielectric" consists of a borax of phosphate solution, the increase in *effective* capacity is phenomenal. Presumably the larger part of nerve capacity is of this general character.[2] It is true that the extraordinary capacity of electrolytic condensers may be due to gas polarization, i.e., to a very thin gas layer as a dielectric at the aluminum anode. However, any ion in solution which is impeded by an interface should participate in its polarization concentration potential, and the particular reactive ion concerned in electrical stimulation of nerve may be so involved. Such a polarization capacity, or "polarizable resistance," in nerve, should be much the larger part of its total capacitative reactance in the circuits encountered.

As to the manner in which this reactance is involved in nerve functioning, it is not necessary to be specific here, except to recognize that stimulation of nerve by an electric current takes place in accordance with the laws governing the charging of a capacity or the polarizing of a membrane (Lapicque, 1926; Bishop, 1928) and that most agents which depress the function of nerve also reduce or abolish the electrical polarization (though not necessarily the polarizability) of its surfaces. This amounts to stating that the biological process of nerve is one intimately involving electrolytes concentrated at its surfaces, the normal function depending both upon the physico-chemical state of the surfaces (polarizability) and the concentration of the electrolytes at them (polarization). These variables are probably not independent of each other, and there is no reason for assuming that the inorganic salts are the only electrolytes concerned.

The irritable apparatus of nerve thus involves the polarization of at least one particular interface in the nerve; however, the total effective

[2] Polarization by hydroxyl ions, a potential source of oxygen ions, or perhaps by some other oxygen yielding ion, is not to be excluded as a possibility, especially in view of the nerve's dependence upon oxidation for its electromotive action, and of the probability that an oxygen reserve exists in nerve, which can be drawn upon during asphyxia (Gerard, 1927; Heinbecker, 1929). The analogy of the iron wire nerve model (Lillie, 1926) is perhaps not far-fetched in this connection.

capacity as measured from leads at the outside of the nerve trunk, includes the effect of polarization by the measuring currents of any interfaces across which the current flows. There is reason to believe that much of the effective capacity of nerve is involved in the polarizability of the myelin sheath, and this cannot be differentiated from the polarizability of an underlying irritable structure at present.

Nothing has appeared in this investigation to require any essential qualification of the core-conductor hypothesis; that is, the results are consistent with a structure for nerve fibers as cylinders of polarizable material containing relatively nonpolarizable electrolytes, although a

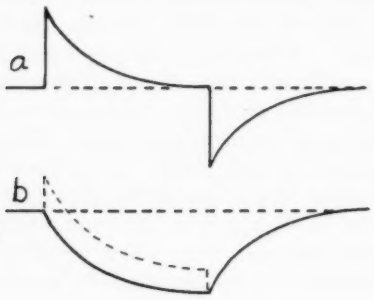

Fig. 2

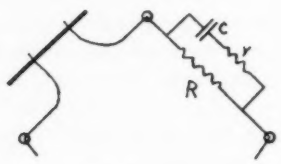

Fig. 3

Fig. 2. Polarization curves from nerve, diagrammatic, from constant potentials of a few sigma duration. *a*, bridge balanced by resistance only after (approximately) complete polarization, measuring direct current resistance. *b*, balanced immediately at make of current, measuring resistance to oscillating current of infinite frequency (instantaneous rise of applied potential). Dotted curve indicates intermediate resistance balance.

small longitudinal component of polarizability in the axons cannot be excluded, and probably exists.

3. *Distribution of the capacity in nerve.* When a galvanic current of short duration (5–10 sigma) is passed across a Wheatstone bridge, one arm of which consists of a uniform length of nerve with electrodes applied to its intact surfaces, and the bridge is balanced with resistance only (C_1, C_2, C_3 absent, fig. 1) the transient polarization picture appears on the oscillograph following make and break of the current. It has the form diagrammed in figure 2 a (see legend). The usual method of measuring nerve capacity is to place a series capacity in the arm of the bridge, opposite to the nerve, and to record the effects of alternating currents of different frequencies. One objection to this technique is that the nerve is obviously not built like the balancing arm, that is, its capacity is not simply in series

with its resistance, but is first, shunted by salt solution, lymph, etc., and second, is probably leaky in itself. The second objection is that this arrangement offers no means of differentiating between *different* components of capacity, if such exist, without involved calculations from data whose meaning is doubtful to begin with.

The galvanic current referred to gives at once the value of the resistance which is nonpolarizable (fig. 2, a) and which thus acts as a shunt or a leak to the capacity, being that resistance which balances the bridge after polarization has been attained. If the bridge were balanced, as is possible (fig. 2, b) so that on make of the current, the curve of polarization rises only gradually from the base line, and reaches a level asymptotically, the value of the resistance so measured corresponds to that for an infinite frequency. To balance out this polarization, capacity with resistance in series is added in parallel to either R_2 or R_3 (fig. 1). The ratio of the effectiveness of the capacity in these two positions will be inversely as the resistance of the corresponding arms R_2 and R_3, (if $R1$ and $R2$ are equal) while the ratio of the effectiveness of the resistance (r) will be directly as those arms. That is, if $R3:R2::100:1$, a capacity across $R2$ must be 100 times that across $R3$ to balance, and the series r must be $\frac{1}{100}$th as great, and this combination of r and c will be unique. It is advisable for reasons of mechanical convenience to utilize both arms $R2$ and $R3$ of the bridge for balancing the nerve at $R4$ with different capacities, because one c required may be a small capacity, and one r is too large for convenient measurement; when $R2$ was utilized, c and r are reported as of the proportional values in $R3$.

If now the nerve's capacity were replaceable by a single condenser shunting its nonpolarizable resistance component, a resistance in series with this capacity would be required such that, in parallel with the nonpolarizable resistance, the result would be equal to the resistance of the nerve for infinite frequency. That is, if R, figure 3, balanced the nerve after it was polarized, as in figure 2a, and the added $r\,c$ balanced the nerve's polarizability, the resistance $\frac{Rr}{R+r}$ ($R + r$ in parallel) would be the resistance at infinite frequency, to give the curve 2 b.

It is found to be impossible to balance the polarization of nerve by one capacity in this arrangement; in fact at least three are required, each with a separate resistance in series. A relatively large capacity with relatively high resistance balances one component (fig. 1, c_1) a smaller capacity with a lower resistance balances a second (c_2) and yet smaller one approximately balances a third (c_3).

There still remains a slow shift of potential lasting from the end of the

10 sigma interval selected for analysis for an indefinite time, similar to the polarization of the calomel electrodes referred to above, but more pronounced for the same current flow. It could presumably be balanced out by one or more very large capacities in series with very high resistances, in parallel with the rest.

The technique of adjusting these values is as follows. A short circuit across the bridge is opened periodically by the pendulum (at *S*, fig. 1) for a 10 sigma interval (*m* to *b*, fig. 4); one capacity and its series resistance are alternately adjusted so that the decremental tail of the curve approaches the base line from behind forward (fig. 4, *a* and *b*; p_1 compensated, p_2 and p_3 left). This is carried as far forward as possible by increasing the capacity and decreasing the resistance, until a diphasicity, or over-compensation in the first part of the record (p' dotted, fig. 4b) just fails to occur, leaving the latter part of the curve coincident with the base line. The process is repeated with a second and a third set of capacity-resistance elements. Slightly better adjustment by trial and error can then be made by throwing a sine current from the induction coil across the bridge (see fig. 10, *A C*, appendix). The shunt resistance R_3 does not require changing during these adjustments, and the later condenser units added do not affect the adjustment of the earlier appreciably. Usually a spike is left at the start of the curve of not over 0.05 m.v. with 30 m.v. applied to the bridge. Its duration is a very few hundred thousandths of a second, a value masked by the amplifier reactance.

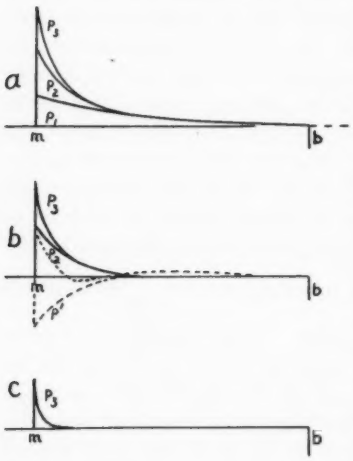

Fig. 4. Diagrammatic analysis of reactance bridge compensation of polarizable nerve. *P1*, *2* and *3* are the summating components of polarization due to galvanic currents, compensated by *c1r1*, *c2r2*, *c3r3* of figure 1, the curves differing by progressively decreasing time constants. See text.

When the circuit is balanced for the make of the current, (break of short circuit) the balance may not be perfect following cessation of current at the end of the 10 sigma interval (at *b*). This discrepancy is presumably a result of differences in polarization at anode and cathode, superimposed upon slight inequality of the size, condition, etc., of the nerve under the two electrodes.

These three components of polarization are probably not distinct, each

one being in itself complex; that is, the bridge is still only approximately balanced. They apparently represent the three main components in a continuous series whose sum total of effect in the nerve is not very different from the sum of these three. The second component is the one whose time course corresponds to the stimulation of nerve by galvanic currents (Bishop, 1928) and this is the more prominent one. The first corresponds more or less to what was previously described as a shift in the base line to which the other component returned upon breaking the circuit after a long duration of current flow (Bishop, l.c., p. 428). The third, which appears as a shock-like escape in some of our published records at start and end of a galvanic current in an approximately balanced bridge, has been considered to be capacity of the apparatus, but is apparently not altogether instrumental, and possibly represents a longitudinal component of polarization.

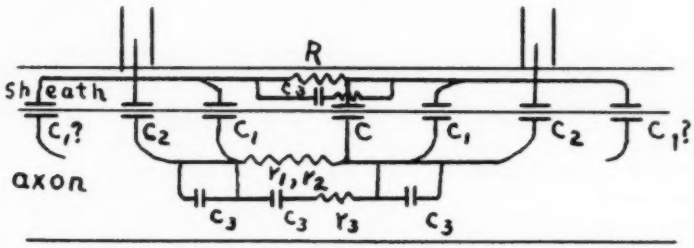

Fig. 5. Schematic diagram of the presumptive locus of polarizable mechanisms inferred to be present in nerve.

With this distribution of capacity in the nerve, it is obvious that when the bridge is approximately balanced with only one capacity, the apparent minimum imbalance will change with the frequency, the higher frequencies reacting less with the "slower" components of the nerve's polarizability, i.e., those whose curves of potential change have the higher time constants. For reasons to be assigned later, the second capacity referred to is considered to represent the transverse polarizability of the nerve under the electrode (fig. 5, c_2); the first, the result of the progressive transverse polarization of the axons in the same manner (fig. 5, c_1) along the interpolar stretch, ("spread" of current, conduction of electrotonus in the older literature etc.) together with concentration changes under the electrodes such as also occur in the electrodes themselves resulting from electrolysis. The third may be longitudinal polarization of connective tissue, etc., which lies around the axons, or of the axons themselves; its amplitude is low and its time constant is small, corresponding to a small and probably a very much shunted capacity in series with a low resistance (fig. 5, c_3). It does not change in value appreciably on killing the nerve under the electrodes,

but is reduced in dead nerve. Typical values of these effective capacities and resistances, assuming this simple arrangement, for frog nerves and roots, are given in table 1 below.

4. *Effect on capacity of killing nerve at different regions.* In the above scheme, one component of capacity is assigned chiefly to the regions of nerve under the electrodes, the other two being predominantly functions of the interpolar region. The evidence leading to this inference follows. Killing under one or both electrodes might be expected to reduce or abolish the local transverse polarization there, while killing between electrodes

TABLE 1

NERVE	DIST. BET. ELECT.	RESISTANCE IN OHMS $\times 10^3$. CAPACITY IN MICROFARADS $\times 10^{-3}$						
		R	C_1	r_1	C_2	r_2	C_3	r_3
	mm.							
Greenfrog sciatic	7.5	48.6	4.4	1430	0.4	394	*	
Greenfrog sciatic	15.5	84.2	2.7	3060	0.3	765		
Opposite sciatic, in ice box overnight	13	77.6	1.0	8350	0.1	1080		
Same, heated at one electrode	13	76.3	1.0	11500	0.08	2400		
Bullfrog IX roots in parallel	9.5	59.8	2.5	1430	0.38	553		
Sensory alone	5.5	83.0	3.0	1720	0.42	1270		
Motor	5.5	86.5	2.8	1310	0.23	680		
Nerve plexus branch adjoining roots	9.5	44.8	3.3	1370	0.40	307		
Crushed under one electrode	9.5	35.7	4.0	1800	0.28	1390		
Greenfrog sciatic	10	67.2	3.0	1770	0.75	610	0.75	129
Injured by current action potential nearly abolished at one electrode	10	65.6	3.05	1770	0.54	910	0.65	129
Same injury repeated	10	63.6	3.1	1770	0.47	980	0.65	129

* In many experiments C_3 was not employed, the residual imbalance remaining as in figure 4, C.

should not alter it, and the reverse should hold for a polarization resident in the interpolar stretch. It is found that when the nerve is killed under one electrode, a new balance is obtained by a reduction in the value of the compensating condenser c_2 and an increase of the corresponding r_2, and no other adjustment is effective. On the other hand, when the nerve is killed between leads, compensation by this element c_2r_2 is little changed, but the other elements must be altered in order to compensate for the decrease of capacity that occurs. When the nerve is killed locally under both electrodes, but not between them, the capacity corresponding to c_2 is nearly obliterated, and that corresponding to c_1 is much reduced, but that compensated by c_3 is still near its original value. These severe injuries

were produced by crushing or by application of hot water, as well as by strong electric shocks.

The specific interpretation of these results involves a consideration of the behavior of the capacity-resistance network of the bridge of which the nerve comprises one arm. The effective capacity of two equal condensers in series (one under each electrode) is one-half that of a single one of the same value, and this rule holds for shunted or leaky condensers also, provided that the resistances in series and in shunt are also equal. Thus at first sight, if the nerve is killed locally at one electrode without altering its ohmic resistance materially, there might be expected a doubling instead of a reduction, of the value of the capacity which, in an opposite bridge arm, would balance the polarizability of the nerve under the other electrode alone. The results, however, of killing by crushing, by heat or by high

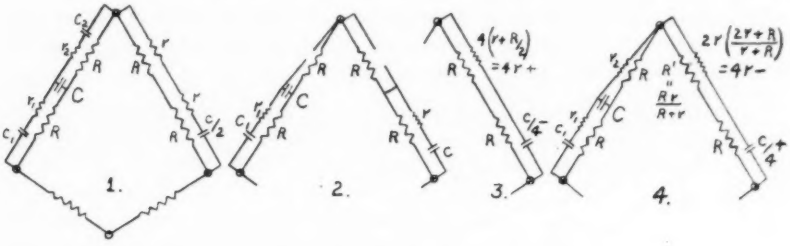

Fig. 6. Bridge diagrams for analysis of meaning of network which compensates nerve in a Wheatstone bridge. Analysis in text and note 3.

frequency current at one electrode is to approximately *halve* the corresponding balancing capacity, while the resistance in series with it must be increased. This effect, however, is assignable, on the basis of the probable character of the nerve circuit, to the effective rearrangement of the resistances upon removal of this capacity.

Reference to the bridge diagrams (fig. 6) indicates that, even though killing induced no change in the ohmic resistance components of the nerve, the removal of the capacity at one end effectively redistributes the resistance with respect to the capacity which remains, and thus alters its effect in the bridge. Let c and r (in fig. 6, 1) be the local capacity and its series resistance, R the shunt, at each electrode, and C the transverse capacity between parallel conductors r and R distributed along the inter polar region. C will be large relative to c, and may be considered for purposes of this argument to act as a by-pass at the middle point, no current flowing through it, so long as the network is symmetrical (the nerve intact). The bridge will balance if the opposite arm contains $\frac{c}{2}$ and $2r$ in series, shunted by $2R$

$\left(\frac{1}{c} + \frac{1}{c} = \frac{1}{c'}, c' = \frac{c}{2}\right)$. Now if killing at one end removed both c_2 and its resistance r_2 (in 2) the remaining $c_1 r_1$, shunted by R through C as a by-pass, would be compensated either by c and r across $\frac{1}{2}$ the resistance of the opposite arm (R), or by $\frac{c}{4}$ and $4\left(r + \frac{R}{2}\right)$ across the whole ($2R$) of the opposite arm, as in 3.[3] What occurs, however, is that r_2 becomes a parallel resistance to R (in 4) which then amounts to $\frac{r_2 R}{r_2 + R}$; R' must therefore be decreased to this value; $\frac{c}{4}$ must be increased correspondingly, and $4r$ decreased; all assuming the C is large compared to c, or is otherwise compensated as a separate component of capacity (c_1, of previous section).

These relationships can be calculated from the equation for shunted condensers; they have been observed in a bridge network in which the nerve was replaced by the mechanical system presumed to represent it, and balanced as arranged in figure 6, 4.

[3] The conditions for the initial current to balance the two arms of the bridge diagrammed in figure 6, 2 and 3, are that their parallel resistances shall be equal, and for the final current that their shunts be equal.

(1) $$R + \frac{Rr}{R + r} = \frac{2RX}{2R + X}$$

and

(2) $$2R = 2R.$$

From (1)

$$X = 4\left(r + \frac{R}{2}\right)$$

If R is small compared to r, X approaches $4r+$, and, by a corresponding computation, c approaches $\frac{c-}{4}$.

For the arrangement of figure 6, 4,

(3) $$\frac{2Rr}{R + r} = \frac{\left(\frac{Rr}{R + r} + R\right) X}{\frac{Rr}{R + r} + R + X}$$

from which $X = 2r\left(\frac{2r + R}{r + R}\right)$.

If $R = r$, this becomes $3r$; if R is small compared with r, as in nerve, X approaches $4r-$, and c approaches $c/4+$, and the shunt resistance $R + Rr/R + r$ approaches $2R-$.

If diagram 4 represents approximately the condition in nerve killed under one electrode, then the resistance $2R$ to balance should have to be reduced to the value $R + \frac{rR}{r + R'}$, the capacity to balance reduced from $\frac{c}{2}$ to somewhat more than $\frac{c}{4}$, and the resistance in series with it increased from $2r$, in the same proportion. Since, in nerve, the resistance which appears as r is large compared to R, R' should be not greatly altered by killing at one electrode, and the result should be to approximately halve the balancing capacity corresponding to the capacity abolished in the nerve, and to double the resistance in series with it. This increase of r_2 and decrease of c_2 and R is what occurs upon *thorough* destruction of the tissue *under one* electrode, by any of the agencies employed, with respect to the balancing unit referred to in previous sections as c_2r_2 (table 1). The effect of partial destruction or less severe injury will be considered below.

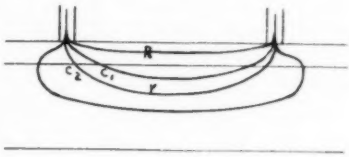

Fig. 7

The capacity in nerve corresponding to c_3, whose charge has the briefest time function, is considered to involve a longitudinal component rather than a transverse one because it is reduced progressively in value as more of the nerve is destroyed. It should theoretically be representable, in case this is true, as a number of capacities in series arranged linearly along the nerve; the value of a compensating capacity might be reduced upon destroying part of these, instead of increasing, for the same reason as was offered for the two transverse capacities under the two electrodes where current enters and leaves the nerve. That is, part of the series resistance is thrown into parallel with the shunt resistance, upon abolition of its respective capacity, the remaining capacity then subtending only a part of the previous shunt (fig. 6). It is more convenient to measure this in the compensating bridge arm as a capacity across the whole arm, instead of across only a part of the resistance of this arm; and in this arrangement a small capacity with high resistance compensates for a larger effective capacity with less resistance in series, since the latter is effective through only a part of the length of the nerve. The values obtained here are thus in a sense equivalents as components of a circuit, but do not represent true values of any actual polarizable structures in the nerve.

The capacity corresponding to c_1, abolished or reduced by killing *between* leads, is inferred to involve the transverse polarizability of the axons in the interpolar stretch, quite as c_2 does that under the electrodes, because it appears reasonable to suppose that transverse capacity distributed along the axons would have such a difference of effect at different regions,

depending on the manner in which current entering at one level distributed itself through the nerve at different cross sections. Figure 7 indicates the presumptive conditions of current flow through the nerve. Considering the membrane C as capacity, R as salt solution, etc., shunting it, and r as the core resistance, for diagrammatic purposes, at regions a and e under the electrodes current flows across the membrane to traverse the core. A part of the current through R however also flows across the membrane between electrodes, except at the central cross-section where all components of current are longitudinal, and the membrane potential is not altered. As the capacity at a acquires an E.M.F., this acts like an increase of resistance in series with r, which causes relatively more current to flow through R, thus inereasing the potential effective across c at b, etc., progressively with duration of current flow, and the capacity then changes potential more slowly than it would if the full final voltage were established at once. Each region must therefore react reciprocally on all others; and the combined curve of counter E. M. F. of all these differential units of capacity under a constant voltage applied between two regions of the nerve, is no longer a simple exponential function, but might be looked upon as the sum of a series of curves, with progressively slower functions. For this reason one can only state that a simple compensating circuit consisting of one capacity, etc., may represent *predominantly* the capacity at one region in the nerve, and does not completely correspond to it, even granting the simplest assumption that can conveniently be made. Also, if the transverse capacity of the axons, etc., were distributed uniformly along the nerve, which it presumably is, its *effect* as measured by a change in the current entering and leaving the nerve at specific levels, must vary in different parts of the interpolar region. Even if the capacity in different localities may be *approximately correlated with* different compensating capacities in a bridge, no one capacity can be exactly equated to one component of the nerve in this arrangement.

The above discussion refers to polarization capacities whose effects are of short duration, when tested with galvanic currents.[4] The ten sigma period chosen for compensation is arbitrary, and further relatively slight but long lasting polarization takes place thereafter. The latter can by no

[4] Further qualification of any arbitrary statement of exact correspondence between capacity components as measured and the polarizability of nerve, is necessitated by the fact that only small currents can be used in measurements, since stimulation may occur at anything above 30 millivolts. With the sensitivity of apparatus obtaining and the small currents permissible, it is possible, for instance, to slightly increase one capacity and decrease another proportionally, with a corresponding change in the resistances, and still leave an approximate balance as before. The limits of such changes however are small, and insufficient to compromise the qualitative statement that a range of capacities with a typical distribution is required to balance the nerve.

means be ignored biologically, since it probably contributes to the current effect referred to as electrotonus, whose manifestations increase progressively with times much longer than those here considered. However, it is not immediately involved in the measurement of reactance to the usual transient currents employed in dealing with tissues, and is thus beyond the scope of this study. On the other hand, it may represent a part of the *injurious* effects of strong electrical currents, considered as exaggerations of electrotonic changes; it presumably passes over into the more or less massive and irreversible transfer or alteration of substances electrolytically, as contrasted with the more transient and promptly reversible ion concentration at the immediate interfaces. It acts analogously, though is not identical with the "stickiness" or residual charge of a Leyden jar or other physical capacity.

5. *Capacity in nerve trunks as compared with roots.* In the bullfrog sciatic preparations it is possible to obtain a pair of roots with the branch of the sciatic plexus to which they run, each having a uniform 2 cm. stretch essentially without branches (ignoring the sympathetic rami). The bullfrog nerve plexus branch is approximately the size of the green frog sciatic in the bifurcated but otherwise unbranched region of the lower thigh. Comparison of these three structures shows no essential difference in their reactances other than that dependent upon diameter; in other words, the distribution of capacity in a nerve surrounded by a heavy sheath is essentially the same as that in a pair of roots of the same length and cross sectional area, surrounded by a very thin sheath. This amounts to stating that the tubular connective tissue sheath surrounding the nervous tissue has a reactance similar to that of other nervous elements surrounding the first. This finding is rather surprising in view of the fact that, for an electrical stimulus bearing a given relationship to threshold response of the fibers, the reactance as reflected in the shock "escape" or distortion is much greater for the nerve trunk than for the roots. It is obvious however that the connective tissue interposes into the current pathway through the axons both series and shunt resistance, polarizable and ohmic, each of which should reduce the current effect at the axons; the effect should vary in fact as the product of the shunt and series resistance effects of the added sheath, or somewhat as the second power of the sheath thickness. The electrical stimuli necessary to reach the thresholds of axons in nerve and root respectively should therefore give artefacts of quite different order of magnitude even if the *distribution* of the reactance were similar in the two structures.

6. *Change in reactance with depression or partial injury by electrical shocks.* If instead of thoroughly killing, electrical shocks from an induction coil are sent through the nerve under one electrode, sufficient only to partially depress the action potential and raise the threshold at that region, a corre-

sponding decrease in the reactance takes place. This decrease, like that from complete destruction of the polarizable structure, is compensated by decrease of only one of the balancing capacities (*C2*) and increase of its series resistance, and the change progresses as progressive injury takes place. The limit is an approximate halving of the value of *C2*, associated with complete destruction of the polarizability under the one electrode, this limit is, however, by no means reached when the nerve is depressed just to abolition of the action potential. The experiments so far are qualitative only.

The procedure is to balance the bridge as previously described to a galvanic current, then by a switch to throw across the bridge make and break

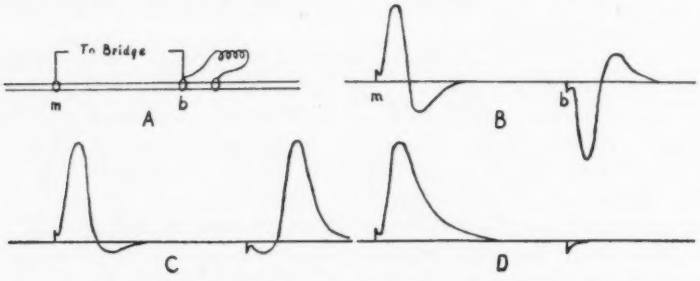

Fig. 8. *A*, arrangement of nerve for recording action potentials and polarizability after injury. Stimulating and measuring currents are passed across the bridge; injurious currents are delivered by the auxiliary induction coil at the right. *B*, *C* and *D*, effects on potential record of progressive injury at an electrode. Make and break shocks are effective at opposite electrodes, *m* and *b*. The action potentials are recorded at one-half their voltage due to shunting by the opposite bridge arm, the record still being 30 mm. high at $\frac{1}{8}$ of the maximum sensitivity of the recording apparatus.

induction shocks at threshold and at maximal intensity. The make shock stimulates at one electrode, the break at the other. Each action potential is diphasic, the first phase being recorded at the site of stimulation, the second after conduction through the length of nerve subtended by the electrodes, and the two processes are mirror images of each other (fig. 8B). Injury produced by a Porter cored coil with 3 volts across the primary, the secondary 3 cm. from the close position, faradizing current for fifteen seconds, in a bullfrog sciatic plexus 8th nerve branch, with 5 mm. between the electrodes of the injurious current, results in depression of amplitude of one phase of each action potential to approximately one-half (fig. 8C). The other phase is recorded as higher than before, since it is less opposed by the depressed phase, the two overlapping at the distance employed. The fact that the second or conducted phase actually increases in amplitude when the first, recorded at the injured point, is depressed, indicates

that few if any fibers have failed to respond to an adequate stimulus at this stage. The threshold at this electrode is raised considerably, however. Such a degree of depression without extinction is accompanied by a material decrease of polarizability under one electrode, as indicated by a decrease in the capacity of *c2* of 15 to 20 per cent to rebalance the bridge.

With a repetition of the injury procedure or with stronger currents extinction of one phase may result, indicating both failure of conduction from the uninjured to the injured region, and failure of response at the injured point; the two circumstances appear to coincide. In this case one whole process drops out and the other becomes monophasic (fig. 8D). A still further decrease of polarizability accompanies this. However, if the injury is only just sufficient to depress the nerve under one electrode to extinction of function, the balancing capacity *c2* does not decrease to the one half of the initial value which follows complete death, either by heating, or by electrical injury from prolonged faradization with the secondary coil close over the primary. A decrease of 20 to 30 per cent of *c2*, or from 40 to 60 per cent in the polariziability associated with the injured region of the nerve, involves failure of response to stimulation.

Nerves so injured do not recover, either in polarizability or in irritability, during two hours. No mechanical effect is visible macroscopically from this degree of injury; microscopical examinations have not yet been made.

Discussion. These bridge measurements of the capacity involved in a segment of nerve traversed by a current entering through uninjured surfaces, do not correspond immediately to specific components of polarizability of the axons. The network employed represents about the simplest one which will effect even an approximate balance; a mechanical duplicate of the nerve's capacity would certainly be more complex, and would display a materially different arrangement. Certain inferences have been drawn as to what general type of arrangement this would have to be; the details of structure are even more indefinite. It has been disappointing that so far no evidence has appeared to differentiate precisely between the polarizability of the critical irritable structure in the axons, and other structures which are presumably polarizable but physiologically not nervous. The indications are however that the polarization which is involved in the time course of stimulation (as indicated by chronaxie, etc.—Bishop, 1928) is not precisely that which corresponds with the *irritable mechanism* alone, but that the composite effect of all the polarizable structures present influences the time course of the change in the irritable element. This complex polarizability determines the time course of the flow of current which stimulates. Under a given potential difference, the fraction of the total quantity of current flowing which goes to stimulating, as well as its time course of flow, are functions of the nerve's effective capacity and resistance; that is, of the reactance of the circuit through the nerve as ar-

ranged on its electrodes. The quantity of this current necessary to effect a stimulation is obviously also a function of the *specific chemical process* to be initiated. The capacity as measured might be termed the *equivalent* capacity, and is of little significance by itself, without consideration of the resistance distribution associated with it.

It would appear that the only way to measure the specific capacity of nerve axons precisely would be to insert an electrode inside the axon, insulate the open ends and measure reactance per unit length between core and external medium. Measuring transverse resistance across nervous tissue (Hermann, 1871) does not give it directly, and it is a question if the capacity of any one structure could be computed from such measurements. Goethlin's computations (1910) from the dielectric constant of extracted myelin are open to the objection, besides the obvious ones concerning structure, that not electrolytic polarizability, but Maxwellian capacity is being computed. Crehore and William's (1913) theoretical considerations appear to involve true capacity of parallel conductors instead of the transverse electrolytic polarizability to current entering the axon to stimulate; these authors, however, concerned themselves, not with measurement of the capacity, but with its possible effect in nerve conduction. Krüger (1928) has measured the polarizable resistance of a stretch of nerve mounted upon electrodes, to oscillating current of different frequencies, in terms of a series capacity, and concludes from his results that the nerve capacity is in reality a shunted polarizable resistance, but gives no measurements of a network that would represent nerve. The change in apparent capacity with frequency observed by him is presumably in part at least to be attributed to the *different* effective capacities which act as if in series with different resistances, the composite reactance having a different apparent value for each rate of oscillation of the measuring current.

The results of these measurements of reactance are quite consistent with the tubular structure assumed for nerve in the core-conductor hypothesis, as Krüger points out for his data. Whatever longitudinally directed reactance there is present is not too great to be assigned to the cell membranes of connective tissue, nodes of Ranvier, etc., that should have a component at right angles to the axes of the fibers. Emphasis may be directed to the circumstance that structures situated at the region where current enters the nerve as a whole may have quite different reactance as measured in the bridge from the reactance of identical structures identically arranged with respect to the nerve's axis, but situated between leads, or in the extrapolar region. Different values of reactance *as measured* may be functions of the same type of structure in the nerve, and vice versa.

After electrical injury by which the nerve is functionally extinguished, further injury by shocks of the same intensity further decreases the polarizability of the injured region. This may mean that certain nonirritable

structures located there are "tougher" than the essential nerve membrane, although finally capable of being rendered nonpolarizable; or it may mean that depression to failure of function precedes complete destruction of the irritable mechanism itself. In view of the depressive but reversible effects of polarizing by galvanic currents of low intensity, the latter opinion would seem the more plausible one.

SUMMARY

The "capacity" of nerve is discussed in terms of the polarizability of its interfaces to ions.

A network of capacity and resistance is described which in one arm of a Wheatstone bridge balances the reactance of nerve in the opposite arm, when the lead electrodes are in contact with intact tissue.

The distribution of polarizability in nerve is inferred to be different from the distribution of the reactance in this network, but to involve more than one component, such that no single unit of one capacity and resistance will compensate nerve in a bridge.

By means of the visible cathode ray oscillograph record it is feasible to measure the reactance of a stretch of nerve mounted on electrodes with a *galvanic current* of short duration, to differentiate qualitatively between its different components of polarizability, and to measure its resistance to direct current, and to alternating currents of (practically) infinite frequency.

The reactances of nerve trunks, and of roots with thin connective tissue sheaths, are found to be similar in distribution. The effect of the sheath is chiefly due to its resistance, in series transversely, and in shunt longitudinally, to the nerve fibers.

The effect of injury under one or both electrodes, by heating, by crushing, or by repeated electrical shocks, is to reduce one component chiefly of the nerve's reactance, with little effect on the others. Injury in the interpolar stretch reduces a different component.

The action potential is decreased to extinction, and the threshold is raised, progressively, through injury by electrical shocks, considerably before the transverse polarizability of the nerve under the electrodes is abolished; further injury by shocks of the same strength then further reduces the polarizability to approximately zero at the region injured.

Certain apparatus suitable for the measurement of resistance and capacity with the oscillograph as a null instrument is described in an appendix.

Appendix. In connection with the foregoing work on nerve capacity, an arrangement of oscillograph, amplifier and interrupter has been employed which differs in some details, though not in principle, from that previously described by Gasser and Erlanger (1922). The requirements for this problem were, that the amplifier should

be as free as possible from reactance, and particularly that the various parts of the apparatus should be brought close together to shorten connections. Wires leading from the interrupter being directly connected through the bridge to the input, the whole had to be shielded to avoid antenna effects. If these shielded wires were long their capacity became appreciable in comparison to that of the nerve.

The pendulum interrupter employed for repetitive recording is, in the form described, neither so flexible nor so precise in timing as the more elaborate rotary interruptor; satisfactory photographs can be taken, however, by means of a hand key, of single deflections at any speed that can be photographically recorded, and of repetitive figures by means of the pendulum interrupter at a sensitivity of 50 meters per volt input, and a speed of up to twenty meters per second.

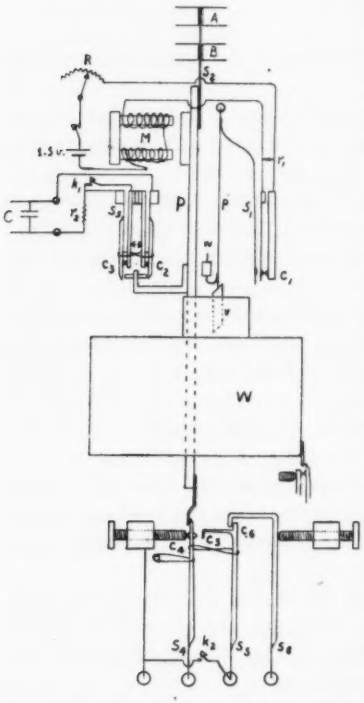

Fig. 9. Diagram of pendulum interrupter operated by a dry cell, to control timing of events recorded on the oscillograph. See text.

The pendulum consists of a bar P (fig. 9) attached to a stiff spring S_2 clamped at A, with a second movable clamp at B to adjust the effective length of the spring for different rates of vibration; a heavy weight W (2–3 kgm.) gives inertia sufficient to obtain fairly constant amplitudes with slightly variable impulses, and therefore the same speed of movement for each beat. The whole is mounted on a heavy cast iron stand. p is a second pendulum, with a weight w, and light wire s, driven by the vane v dipping into a bath of heavy oil which rides on W. Due to the inertia of w, this secondary pendulum will lag behind P in phase, by an adjustable interval. Thus it can be made to close C_1, which may be a mercury drop with which s makes contact, only after P has started on the return swing, and keep it closed for some time. The effectiveness and constancy of this device is such that one dry cell applied to M through several ohms resistance (R) will keep the pendulum swinging regularly while doing considerable work on the various contact keys. A 100 ohm resistance r_1 across C_1 takes care of surge from the inductance in this circuit so that it does not disturb the amplifier even when the leads to the input are very short. It may be operated within 20 cm. of the amplifier input.

Two keys (*C2*,*C3*) connected in series, made of small clock spring with reinforcing ribs on their backs, serve to close a short circuit through r_2 of 100 ohms discharging the condenser C of the oscillograph X axis circuit. An insulated bar moving with the pendulum P allows both keys to be closed in the middle position of P, but opens one or the other when displaced. Energy is applied to P while moving to the left, the critical events occur during the opposite swing, when *C2* is opened. This opening starts the charge of C which moves the oscillograph beam along the X axis. If con-

tact *C2* alone is operative (*k1* closed) the spot remains in the zero position for one half the cycle, which blurs the photographic record. If both *C2* and *C3* operate, the spot starts moving twice during each cycle, once to write the record (*C2*) and again to write an undistorted base line (*C3*) on the return swing of *P*, and may be made to pause at the start of the line for a short interval only, by adjusting the positions of the two keys. A rubber band serves to damp the vibrations of these contacts. All keys have platinum points. The spot is deflected to the right, off the oscillograph screen, during most of the interrupter cycle; the condenser *C* is then charged (see below) and no current flows from the battery.

The lower end of *P* serves to operate keys in other circuits. *S4*, *5* and *6* are all springs, damped by rubber bands. As S4 moves to the right, a short galvanic current can be obtained by using *S4*, *C4*, *C5*, *S5*, as a short circuit, opened during passage of *S4* across the adjustable gap, or make and break induced currents are obtained by sending the primary currents through other keys not diagrammed. All contacts are of platinum. The apparatus operates at a frequency of 2 to 10 per second, and the different circuits to be synchronized (*C2* and *C4*) are opened at a fairly constant interval, as long as the keys open near the middle point of the arc of the pendulum. The interval *C4–C5* is less constant if wide, due to slight variation in the speed of the pendulum from beat to beat. Repetitive deflections superpose closely enough to take sharp photographic records at the rate of movement of the oscillograph beam of 20 meters per second.

The oscillograph beam is deflected by the charging of a condenser *C* (0.02 to 1 mf. variable) connected in parallel to the deflecting plates, through a vacuum tube plate circuit working at current saturation. The record starts when the interrupter opens the short-circuit across the condenser. A shield-plate tube gives a more linear charge than any other tube tested, though even of this, only the first portion is approximately linear. This tube is operated by 2 dry cells for *A* current, and the speed of record is controlled both by a positive bias to both grids and by the filament rheostat, or by varying the condenser.

Time is measured on the oscillograph screen by stepping off with dividers an interval given by a 100 or 250 per second electrically driven Zimmermann fork. This need not be connected to the apparatus, but records by induction through the air. An interrupter cannot be synchronized exactly with this for photographing, but it can be measured precisely on the screen, or a single deflection can be photographed. Plate current supply is drawn from the 180 and 315 volt battery terminals, and the non-linear late portion of the condenser charge passes off the oscillograph screen. All high voltage wires leading to the interruptor and thus passing into proximity with circuits leading to the amplifier are thoroughly shielded.

The oscillograph is mounted slightly inclined to the perpendicular through the vulcanite top of a magnetically shielding box, and the controls are placed on this vulcanite plate. The amplifier contains three shield-plate tubes operated at 315 volts on the plates, with 350,000 ohm coupling resistances. The amplification is potentially 150,000, but is usually cut down in operation by a potentiometer between second and third panels to 30 or 60 per cent of this, or further by a 600,000 ohm potentiometer at the input. The amplifier is mounted on a heavy iron plate supported on coiled springs damped by rubber sponges, the whole resting on a large cement block supported on cork bricks, and the tubes are suspended on rubber bands inside a shield. It operates on the fourth floor of a laboratory building adjacent to 110 volt D. C. light lines, which however run in grounded shields. At a sensitivity of more than 50 meters per volt, building vibrations from passing traffic and noises transmitted through the air disturb the amplifier, but do not preclude its use as a null instrument for recording a bridge balance.

A single *A* battery supplies current to the three amplifier tubes. One 315 volt bank of heavy duty dry cells operates the amplifier, oscillograph and X-axis vacuum tube condenser charger in parallel without perceptible interference, a 4 mf. ballast condenser being connected across the battery terminals. Since there is often occasion to operate the oscillograph at 400 volts, the 110 volt house current is added to this battery, after passage through a 60 milli-henry choke ballasted by a large electrolytic condenser. This current cannot be used on the amplifier. The negative pole of the B battery being grounded, the anode of the oscillograph is 315 volts +; the cathode is made 90 volts—to the ground by the filtered power-line supply. All parts of the apparatus are grounded at the negative leads to a water pipe and all shields to a sewer pipe. The whole apparatus can be arranged on a space of ½ square meter.

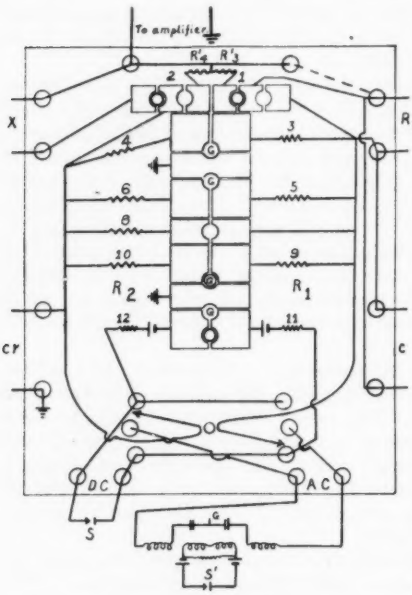

Fig. 10. Symmetrical bridge box diagram for nerve recording. The interrupter is connected at S and S′, for direct or alternating currents through the selector switch. Small dry cell batteries to give direct current are connected into the bridge arms in series with the high resistances 11 and 12, through the switch; these resistances form with the other coils in the corresponding arms a pair of potentiometers. The binding posts *D C* allow other sources of current to be applied when using the bridge to balance out the stimulus record. See text for further details of operation.

Condensers of 0.1 m.f. capacity coupling the amplifier panels, with 2 megohm grid leaks, serve to record transients up to 50 sigma without significant falling off of the potential. Two m.f. condensers soldered in parallel with these as occasion demands allow potential deflections of several seconds' duration to be recorded satisfactorily, but add to the reactance as distributed capacity.

The capacity bridge proper is made up as a plug box (fig. 10), the resistances within the box being reverse layer wound coils (Leeds & Northrup). At *R3* is connected a 100,000 ohm Leeds & Northrup 4-dial box made up of these coils, and at *C3* and *C2* resistances and capacities in series. 1 and 2 are 10,000 ohm check resistances for arms *R3* and *X*. 3 and 4 are of 2,000 ohms each, both connected to ground by one plug, in arms R1 and R2 of the bridge. 5 and 6, 1,000 ohms each, can be connected parallel to 3 and 4, similarly 8, 9 and 10, each 500 ohms, while 11 and 12, 48,000 ohm Lavite 38 *A* resistances, in series with two small flashlight dry cells of equal voltage, can be plugged either in series or both to ground. It is obvious that 3, 11, the ground connections through the switch and one battery form a potentiometer applying $\frac{2,000}{50,000}$ x 1.5 v. in arm 1 of the bridge; likewise for arm 2. Since the nerve resistance in *X* is high compared to 2000 ohms, it will then have about 60 millivolts applied to it. By varying the shunts across these arms the effective voltage may be varied.

The three-way switch is a reversing switch in the extreme positions connecting the constant potential into the bridge. The leads *D C* to the interrupter serve as a short circuit except when the contact is periodically opened. The middle position of the switch leads alternating current into the bridge via *A. C.*

The coil (described previously, Bishop, 1927) has two symmetrical secondary windings of 600 ohms each, through which the current can be varied or reversed by turning the primary. Varying the capacity placed between these coils varies over a wide range the frequency of the oscillations, a decrementing series of otherwise very pure sine waves resulting at make and break of the primary current. The bridge can be checked for symmetry by comparing the effect of a ground at *G* between *R1* and *R2*, with a ground at any other point marked *G*, *X* being replaced by *R'4*, *R3* set at 10,000 ohms or replaced by *R'3* and the capacity boxes set at zero.

The resistances *r*, of 500,000 ohms maximum, each consists of a circular graphite line on a fiber disk, with a sliding contact made of a soft pencil lead mounted on a radial arm. Such a variable high resistance cannot be accurately calibrated, but may be measured each time its setting is changed; it has no appreciable inductance and extremely low capacity, and by varying the width of the graphite line, may be made from 10,000 to 10,000,000 ohms maximum resistance.

The check resistance 1 is arranged to be plugged in series with the arm *R3* of the bridge. The nonpolarizable electrodes employed have a resistance, with thin yarn wicks, of 9 to 10 thousand ohms. This is in series with the nerve just as the corresponding 10 thousand ohm resistance is in series with the capacity-resistance network that balances the nerve. This network will have appreciably different values for its components, depending on whether the capacities are connected across the whole arm *R3*, or only across that part of it corresponding to the nerve resistance without the electrode resistance.

With the visible and distortionless oscillograph record as a detector of the balance of the bridge, transient distortions and noises that might interfere with other types of recording, (for instance, with rectification of oscillating current) may be ignored in the record, and shielding need not be particularly thorough. Metal boxes serving as shields are grounded, but covers may be dispensed with, allowing convenient manipulation. One hundred ten volt lights may be employed within ½ meter of the apparatus if their lead wires are shielded by twisting a grounded pair of wires in with the leads, and soldering them to the reflectors.

Besides serving as a capacity bridge, this apparatus provides a convenient means of balancing out the stimulus distortion from the nerve action potential record. The stimulating leads are connected in one arm of the bridge, the separate pair of recording leads then passing directly to the oscillograph amplifier.

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ABSORPTION AND EXCRETION OF ARSENIC, BISMUTH AND MERCURY: EXPERIMENTAL WORK ON THE COLON[1]

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So much work has been done on the absorption and excretion of arsenic, bismuth and mercury that it would seem that little of interest could be added. However, analysis of the literature reveals that the kidneys and liver have received the largest share of attention. Studies of gastro-intestinal absorption are few and not selective. Not a single observation is cited of absorption and excretion in the isolated colon. It would seem that if this organ could be removed from the course of the normal fecal stream, yet leaving its circulation and nerve supply intact, studies might be carried out on the true nature of the colon as an absorptive or excretory organ or both. With this in mind, the colons of a number of dogs were isolated, as will be described. The objection might be raised that the state produced by removing the colon from its usual situation is so abnormal as to invalidate the results. It must be remembered, however, that the blood supply and nerve supply, as well as the wall and lining of the bowel and even the serosa, remain intact in such a maneuver. The claim might be made that the results obtained in this way would not be applicable to the study of the physiology of the colon of man. Nevertheless, results so obtained in regard to excretion and absorption from the colon should be clean cut and significant.

It would seem that the colon would be of utmost significance in the study of the fate of any substance introduced into the body, a part or all of which might be excreted. Furthermore, the colon might play an important part in absorption and reabsorption of many substances. All reported observations of intestinal absorption and excretion concern analysis of feces or analysis and histochemical studies of tissues after death.

Thus, Brown and Osterberg found that following administration of treparsol (formin derivative of meta-amino-para-oxyphenyl-arsenic acid)

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by mouth, arsenic was found in the urine and feces, but much greater amounts of it in feces. Kolls and Youmans found the liver to be more important than the kidneys in excretion of arsphenamine and neoarsphenamine; about three-fourths of the arsphenamine injected had left the blood stream in a few minutes after the completion of an injection, and the drug was stored in the liver, spleen, kidneys, lungs and in the cardiac and skeletal muscles.

Kuroda stated that arsenic is excreted by the kidneys, bowel and skin. He found the bowel to be important in excretion of arsenic, and his study concerned the rôle of the liver in relation to this. He made gall-bladder fistulas in dogs, tied the common bile-duct, injected arsenic subcutaneously and analyzed bile and feces. He also analyzed urine. He found between twenty-five and thirty times as much arsenic was excreted in the urine as is in either bile or feces. There was direct excretion of arsenic by the bowel, as evidenced by the finding of arsenic in the feces, but the amount excreted in the bile was ten times greater than that excreted directly through the bowel. He found, further, that arsenic acid was reduced to arsenous acid, in the liver. He used the following compounds of arsenic: sodium arsenite, 54.81 per cent arsenic; neosalvarsan, 20 per cent arsenic; sodium arsenate, 24.17 per cent arsenic; sodium cacodylate, 33.9 per cent arsenic; atoxyl (sodium arsanilate) 24.1 per cent arsenic, and elarson, 10.6 per cent arsenic.

Dutcher and Steel found that 71 per cent of arsenic injected was eliminated in the urine, none in the feces, and that the rest was reabsorbed and distributed in various tissues: muscle, liver, intestine, blood and skin. Cornwall and Myers observed the relative distribution of arsenic in the liver, spleen, and kidneys of rabbits following the intravenous administration of silver arsphenamine. Fordyce, Myers, and Rosen, after intravenous administration of neoarsphenamine, silver arsphenamine, and tryparsamide N.N.R. in rats found arsenic primarily present in the liver, spleen, kidneys and blood. Myers and Cornwall, four days after the administration of silver arsphenamine, found twelve times as much arsenic in the spleen as in the liver.

From the work of many students one learns that mercury is excreted much slower than arsenic, but by similar routes. Thus Cole, Gammel, Rauschkolb, Schreiber and Sollmann were able to recover only one-sixth of the mercury injected intramuscularly in man in the form of organic mercurials and one-sixth from the urine and feces. From a later publication by these authors it is learned that the rate of urinary excretion of mercury in man is high for the first two to four hours after injection, and that thereafter the rate falls rapidly. In the first two days, 5 to 15 per cent of the amount of mercuric salicylate injected is excreted, and at the end of the first week, 85 to 90 per cent of the mercury injected is not excreted. Furthermore,

the amount excreted in the feces is only about half of the amount excreted in the urine.

Hofstadt found the urinary tract to be the chief route of elimination of mercury. Christeller and Sammartino noted that the organs chiefly concerned in excretion of mercury were the kidneys, liver, and lungs; less was excreted by the colon and spleen, and occasionally some was found in the pancreas, cardiac muscle, and central nervous system. Lieb and Goodwin found that mercury also was excreted by the gastric mucous membrane and advocated gastric lavage as part of the treatment of mercurial poisoning, no matter how the poison had been introduced into the body.

It has been demonstrated repeatedly that pathologic lesions of various organs occur following the administration of mercury. The principal and most serious of these lesions seem to be in the kidney. Burmeister and McNally showed that such lesions might appear in the kidneys of dogs as early as five minutes after the introduction, by stomach tube, of mercuric chloride in amounts of 0.12 to 1.5 grams for each kilogram of body weight and that the severity of these lesions in the kidneys and liver would increase during the first twenty-four hours after injection. The other lesions frequently mentioned are in the colon, which these investigators find appear later, not before changes are found in the liver, and at least not before three or four hours after the onset of intoxication.

Elbe found, in squirrels, that within six hours after administration of poisonous doses of mercury edema, preceding necrosis, begins in the tips of the folds of the mucosa of the cecum. Necrosis is complete in twelve hours. He felt that the toxic substance causing this was necessarily transported by the blood stream. Gutman found that the chief injuries were in the intestinal tract and kidneys. In both large and small bowel ulceration, infiltration, and swelling appeared. Lesser injuries appeared in the liver, spleen, suprarenal glands, and central nervous system. He noted that, under like circumstances, pathologic changes following the administration of mercury, in whatever form and by whatever route, are approximately identical.

Baldwin found that mercurochrome-220 soluble N.N.R was excreted through the mucosa of the colon and described a constant ulcer on the median wall of the ascending colon of rats in which mercurochrome was injected subcutaneously. There was also constant "catarrhal colitis." Baldwin believed that these changes indicated that mercurochrome was excreted through the mucosa of the colon. There is the possibility, however, that some toxic product formed in the body in the presence of mercurochrome may have caused these ulcers.

Many reports in the literature indicate that bismuth is excreted far more slowly than arsenic or mercury. Memmesheimer found that the excretion

of bismuth by kidney and bowel was slow and steady and that much more was excreted by the kidney than by the bowel. He concluded that there were certain bodily depots wherein bismuth was held for long periods. Chura also found that the chief excretory routes were through the kidneys and intestine.

Leonard determined the toxicity and the rate and amount of urinary elimination after intramuscular injection of a great variety of compounds of bismuth. He found that the larger the dose, the slower was the rate of elimination and the smaller the amount excreted. He noted that bismuth thiosulphate could not be borne in large doses. He also found that bismuth was fixed particularly by certain tissues, namely, the kidneys, liver and lungs, but that some was to be found in all organs. Mehrtens and Hanzlik's observation that bismuth acts as a diuretic would suggest that bismuth is excreted in the urine.

A most significant study is that of Califano, who found that bismuth was held in the reticulo-endothelial system and that the parenchymatous cells remained relatively free of it; that when bismuth and vital dyestuffs are injected simultaneously some cells of the reticulo-endothelial system take up one substance, other cells take up the other and in some cells one can show the presence of both, in different parts of the cell.

Judging from these observations, it seemed that other phases of the absorption, excretion, and final disposition of these drugs would bear careful study. Our part in this experimental work, so far, has been concerned with the function of the colon of the dog in the final disposition of arsenic, mercury and bismuth.

Method of investigation. Under anesthesia and strict aseptic precautions the colon of the dog was isolated in the abdominal cavity. The ileum was sectioned at the point of termination of the longitudinal blood vessel. The large intestine was sectioned as near the rectum as was anatomically possible. The proximal end of the large intestine was closed. The proximal end of the ileum was anastomosed end to end to the distal end of the large intestine, thus maintaining the continuity of the intestinal tract. The loop of ileum which remained attached to the large intestine was resected with the exception of the terminal 6 cm. The latter was pulled through a stab wound and drained to the outside. The abdomen was then closed in the usual manner. This left the colon isolated intra-abdominally with the ileac end as a stoma for instillation of various medicaments. When healing was complete, the ileocecal valve was near enough to the abdominal wall to fit snugly over a flanged glass tube. This tube was prepared to fit water tight and to its end was attached a small rubber tube with a clamp. The tube could be removed and inserted at will. The glass tubes were fitted in place, and the drugs were instilled, each into a series of dogs, and urine and feces were collected at stated

intervals. Also the loop of colon was washed out to determine the amount of unabsorbed residue. In figure 1 is shown the relation of the structures described in an animal which died after intravenous injection of bismuth. The glass tube described is also shown (fig. 2).

The three dogs also were injected intravenously and the urine, feces, and colonic washings were analyzed at stated intervals. Furthermore, these three drugs were given by mouth and similar periodic observations were made.

The determinations of arsenic were carried out by the electrolytic method of Gutzeit as described by Osterberg. The determinations of bismuth were carried out by the method described by Leonard. This procedure

Fig. 1. Relative position of the isolated colon to the reëstablished intestinal tract.

Fig. 2. A tip for instilling fluid into the intestine.

was satisfactory for the analysis of these biologic materials. In the analysis of feces it usually was necessary to centrifugalize the final solution after treatment with potassium iodide in order to remove the precipitate which was present. This did not affect the final result as the precipitate was white and did not carry down any of the yellow color. The determinations of mercury were carried out essentially by the procedure described by Booth, Schreiber and Zwick, and in a few instances these results were coördinated with electrolytic deposition of mercury. The procedure as described by these authors has not been entirely satisfactory in our hands and in many instances we believed that the results were somewhat low so far as the mercury content was concerned. However, for the purposes of our experiment it has served well, since in this contribution we are con-

cerned with the qualitative aspects of the absorption and elimination of the metals studied rather than with the quantitative aspect.

COMMENT. The results of these methods of study of function of the colon seem clear cut and decisive.

Arsenic in the form of neoarsphenamine is readily absorbed by the colon and the rate of absorption is directly proportional to the time of retention of arsenic in the isolated colon. Arsenic in the form of neoarsphenamine

TABLE 1

*Absorption of arsenic in neoarsphenamine from the isolated colon**

ANIMAL	TIME AFTER INSTILLATION	ARSENIC IN CATHETERIZED URINE	ARSENIC IN URINE	ARSENIC IN URINE AND FECES	ARSENIC IN FECES	ARSENIC IN COLONIC WASHINGS
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	5 hours	1.1				1.8
	18 hours			14.6		
	1 day		4.1		6.5	0.37
	2 to 5 days		7.1		10.0	
2	5 hours	40.5				80.0
	12 hours			43.0		
	1 day		3.0		9.0	0.25
	2 to 5 days		5.5		40.0	
3	5 hours	None				None
	18 hours			43.6		
	1 day		35.0		29.1	0.07
	2 to 5 days		10.6		37.5	
4	7 hours	2.2				5.0
	30 hours			39.2		
5	1 day		5.1		16.2	0.03
	2 to 5 days		5.2		30.0	

* Nine-tenths gram of neoarsphenamine was instilled into the colon of each animal and allowed to remain for five hours; the colon was then washed repeatedly by aspiration of water.

and in the form of treparsol is not excreted by the colon at all, or else only in minute quantities (tables 1 to 3).

Mercury in the form of mercurochrome is not absorbed by the colon. Mercury in the form of mercurochrome and of metaphen N.N.R. when injected intravenously, is not excreted by the colon in the first four hours after injection. This is indicated by analysis of the product of washings from the colon performed every half-hour after the intravenous injection of mercurochrome. That mercurochrome is quickly absorbed and excreted elsewhere in the gastro-intestinal tract is suggested by the fact that dogs

sometimes had emesis of intensely red material immediately after intravenous injection of mercurochrome. Mercury in the form of metaphen, when given by mouth, is not excreted by the colon.

Most of the results of administration of mercury are given in tables 4 and 5. One additional animal received, by stomach tube, 10 cc. of solution of metaphen, 0.2 per cent, and feces and urine were collected for seventy-two hours. The isolated colon was washed out at the end of six, eighteen,

TABLE 2

*Excretion of arsenic following intravenous injection of neoarsphenamine**

ANIMAL	NEOARSPHENAMINE INJECTED	TIME AFTER INJECTION	ARSENIC IN URINE	ARSENIC IN FECES	ARSENIC IN COLONIC WASHINGS
	mgm.	*days*	*mgm.*	*mgm.*	*mgm.*
1	Control	1	0.002	None	None
		2	1.5	None	None
	450	1	6.8	14.0	0.006
		2	2.7	10.3	0.010
		3	1.7	11.2	
2	450	1	3.7	8.6	0.006
		2	9.1	20.3	0.01
		3	3.0	15.6	
3	450	1	5.5	0†	0.11
		2	7.5	0	0.06
		3	0.8	0	
		8	0.1	0	0.005
	60	15	18.0	60.0	0.015
		16	0.55	1.6	None
		17	None	19.0	None

* The indicated amount of neoarsphenamine was injected, the colon washed out with water by aspiration twice daily and the excreta collected for analysis.

† 0 as used in these tables = no specimen or no analysis.

twenty-four, forty-eight and seventy-two hours. From the feces, 7.1 mgm. of mercury were recovered, from the urine, 2.3 mgm., and from colon washings, none.

Bismuth was used in the form of solution of sodium tetra-bismuth-tartrate. It was found that bismuth is absorbed and excreted by the colon. When the solution is given by stomach tube bismuth is excreted by the colon.

In tables 6 and 7 are given the results of most of the experiments with bismuth. One other animal was given, by deep intra-muscular injection,

10 cc. of solution of sodium tetra-bismuth-tartrate, 10 per cent, and excreta were collected for seventy-two hours. From urine and feces, 106.9 mgm. of bismuth were recovered and from colon washings 95.0 mgm.

Our principal interest in this study lay in determining whether or not there was absorption and excretion of the substances mentioned. We were not greatly interested in quantitative results or in amounts excreted by various routes, hence feces and urine, in many instances, were pooled for

TABLE 3

*Excretion of arsenic following the administration of treparsol by mouth**

ANIMAL	ARSENIC IN URINE AND FECES	ARSENIC IN COLONIC WASHINGS
	mgm.	*mgm.*
1	207.5	0.25
2	218.7	None
3	125.0	None

* Dogs 1 and 3 were each fed one tablet of treparsol in meat daily for three days. The colon was washed out twice daily and the urine and feces collected for five days. The total arsenic administered was 220 mgm.

Two other dogs kept on the same diet but without treparsol showed only traces of arsenic in the excreta.

TABLE 4

Absorption of mercury in mercurochrome from the isolated colon

ANIMAL	SOLUTION INJECTED*	MERCURY IN CATHETERIZED URINE AFTER 6 HOURS	MERCURY IN URINE AND FECES AFTER 24 HOURS	MERCURY IN COLONIC WASHINGS
	cc.	*mgm.*	*mgm.*	*mgm.*
1	5	None	None	11.0
2	10	None	None	8.0
3	10	None	None	4.8

* One per cent solution of mercurochrome was instilled into the isolated colon.

convenience in analysis. The isolation of the colon and shunting of the fecal stream around the isolated portion would seem a very efficacious method of careful evaluation of actual absorption and excretion of various substances.

One might speculate on the nature and relation to mercury of lesions of the colon appearing after intravenous injection of mercurials as observed clinically and in such experiments as those by Baldwin. Perhaps the renal lesions predispose to the formation of some toxic substance which, in turn, by its absorption or excretion, causes the lesions in the colon. However, the theory of local irritation without necessarily either absorption or excretion of the drug would seem most likely to accord with fact. These

experiments suggest that the drug like treparsol, as in the treatment of amebiasis, acts not by excretion but perhaps by local effect.

TABLE 5

Elimination of mercury following intravenous injection of organic mercurial compounds

ANIMAL	MATERIAL AND QUANTITY INJECTED	MERCURY IN URINE		MERCURY IN FECES	MECURY IN COLONIC WASHINGS OBTAINED EVERY 30 MINUTES FOR 4 HOURS
		Four-hour specimen (catheterized)	Twenty-four hour specimen		
		mgm.	*mgm.*	*mgm.*	
1	Metaphen, 12.5 cc. of 2 per cent solution	2.0	2.0	7.5	None
2	Mercurochrome-220 soluble 8 cc. of 1 per cent solution	2.5		8.4	None
3	Mercurochrome-220 soluble 5 cc. of 1 per cent solution	1.4		4.6	None

TABLE 6

*Absorption of bismuth in sodium tetrabismuth tartrate by the isolated colon**

ANIMAL	SOLUTION INJECTED	BISMUTH IN URINE	BISMUTH IN FECES	BISMUTH IN COLONIC WASHINGS
	cc.	*mgm.*	*mgm.*	*mgm.*
1	10	15.6	66.7	49.4
2	10	6.0	47.5	8.9
3	8	34.7	118.5	7.1

* The solution of sodium tetrabismuth tartrate (containing 70 per cent bismuth) 5 per cent, was instilled into the isolated colon and allowed to remain four and a half hours. The colon then was washed out and the excreta collected for forty-eight hours.

TABLE 7

*The elimination of bismuth following the administration of sodium tetrabismuth tartrate**

ANIMAL	BISMUTH IN URINE	BISMUTH IN FECES	BISMUTH IN COLONIC WASHINGS
	mgm.	*mgm.*	*mgm.*
1	5.6	154.0	28.5
2	27.8	38.0	26.2
3	40.2	87.5	42.4

* Ten cubic centimeters of solution of sodium tetrabismuth tartrate, 5 per cent, was given by stomach tube. The urine and feces were collected for forty-eight hours and the colon washed out at the twenty-first, twenty-ninth and forty-eighth hours.

We believe that the methods here employed offer a useful means of studying function of the colon.

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XIX. THE ARTERIAL BLOOD PRESSURE AND THE BLOOD FLOW IN SKELETAL MUSCLES IN UNANESTHETIZED CATS AS INFLUENCED BY THE INTRAVENOUS INJECTION OF EPINEPHRIN

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Macdonald and Schlapp (1) published a report in 1926 to the effect that the fall in blood pressure produced by small doses of "adrenalin" occurs only during anesthesia by ether or urethane and is not observed in decerebrated animals from which the ether has been discontinued for some time prior to the injection. Their results were confirmed by Vincent and Curtis (2) who believed they showed that minute doses of "adrenin" injected into the circulation produce a fall in blood pressure only when the animal is under the influence of certain anesthetics; ether, chloroform, chloralose, urethane and possibly others. If, in pithed or decerebrate animals the anesthetic is eliminated, the depressor effect can no longer be observed.

Dragstedt and Wightman (3) and Dragstedt, Wightman and Huffman (4) studied the effects of dilute solutions of epinephrin slowly injected intravenously in unanesthetized and morphinized dogs. They believe small doses of epinephrin 0.2 to 0.4 cc. per kilo body weight of a 1:1,000,000 solution per minute cause not a depressor but a pressor response. The total volume of fluid injected each time varied from 4 to 10 cc. of a 1:1,000,000 solution. They used dogs which, as experimental animals, are less prone than cats to give the depressor responses to epinephrin. The records presented in the second article (4) are reduced to such an extent that they are difficult to interpret. If, however, they agree with the records presented in the first article, which are apparently not reduced appreciably the rise in blood pressure would seem to occur only during the time in which the injection is being made and immediately upon cessation of the injection the blood pressure falls below normal.

Gruber (5) was unable to confirm the findings of Macdonald and Schlapp (1) and Vincent and Curtis (2). Epinephrin in small doses was observed not only to produce at times a fall in blood pressure in decerebrate cats in which the anesthetic had been discontinued for $\frac{1}{2}$ to $3\frac{1}{2}$ hours, but also to produce an increase in the blood flow leaving a striated muscle during the fall in blood pressure indicating a local vaso-dilatation. The depressor

response was obtained usually only after the first injection but occasionally also following the second or third. In these later injections the falls were invariably smaller than those following the first injection. In some instances the only result of the injection was the appearance of Mayer's curves in the blood pressure record. Experiments showed that the pressor response to small doses of epinephrin was increased, however the depressor effect was not entirely lost.

Recently Dragstedt (6) published another article in which he came to the conclusion that the minimal effective dose of epinephrin on sustained administration in the unanesthetized dog produces only pressor effects and the depressor response to epinephrin in anesthetized animals is an abnormal response mediated at least in part by the anesthesia. He further concludes that there is no reason to suppose from his experiments that the suprarenals are not normally and continually secreting epinephrin in amounts sufficient to modify the vascular bed, and that there is reason to believe that an augmentation of secretion which is easily conceivable would have an hemodynamic effect.

Hoskins, Gunning and Berry (7) demonstrated that "adrenin" produces active vaso-dilatation of skeletal muscle vessels and vaso-constriction of the vessels of the skin. Hartman and McPhedran (8) likewise showed that epinephrin in weak solutions caused dilatation of striated muscle vessels. Gruber (9) was unable to obtain vasodilatation in skeletal muscles the nerves of which had just been cut. His results were confirmed by Hartman and Fraser (10) and by Dale and Richards (11). Nor was he able to obtain dilatation of the blood vessels in active striated muscles the nerves of which were cut and stimulated at a rate favorable to vaso-dilatation. Gunning (12) studied the effect of large doses of adrenalin upon the vessels in skeletal muscle and noted vaso-constriction in all instances. Erlanger and Gasser (13) question the possibility of adrenalin causing dilatation of the vessels of skeletal muscles and conclude from their results that "vaso-constriction of both the somatic and the splanchnic areas is the main if not the only effect of the continuous injection of adrenalin." They used, however, extremely large doses, 6 to 11 cc. of a 1:1000 solution, much larger than was used by any of the above observers, who noted vaso-dilatation. The constancy with which 0.1 to 2 cc. of a 1:100,000 solution of adrenalin slowly injected produces vaso-dilatation in muscles in which the nerve is not injured or in which the nerve had been cut and time, two to ten days, allowed for the vessel to regain its tonus (14) leads one to believe that the methods used by the various experimenters were fairly free from "ambiguities" and vaso-dilatation is as much a characteristic action of adrenalin in weak solutions as vaso-constriction is in large doses. Dale and Richards (11) not only confirmed the finding of Gruber but in addition noted that only a few hours were necessary for the recovery of the dilator

response of muscle vessels to epinephrin following the section of the nerve, instead of days as noted by the latter observer.

Since apparently no investigation has been carried out to determine the effect of epinephrin upon the blood vessels in skeletal muscle in unanesthetized animals, it was decided to find out whether epinephrin in dilute solutions caused dilatation of the vessels in skeletal muscle in unanesthetized animals as it does in decerebrate and anesthetized ones.

The effect of epinephrin upon the blood pressure in unanesthetized cats was also studied.

METHOD. Only large healthy cats were used in these experiments. The femoral artery and vein were exposed, under ether anesthesia. The vessels were covered and separated from each other by sterile vaselined gauze after which the small skin incision was sewed up. The animal was then permitted to come out from under the anesthetic. In those animals in which the effect of epinephrin was studied upon the blood flow in skeletal muscles, the spinal cord was sectioned in the lower thoracic region and while the animal was still under ether anesthesia, both femoral veins and one femoral artery were isolated from their surrounding fascia ready for cannulation. Four to eighteen hours later the animal was placed on the table and the stitches in the skin wounds and the gauze from around the vessels removed. Cannulas were placed in the exposed blood vessels. The one in the femoral artery was attached to a mercury manometer for recording blood pressure, the one in the vein on the same side was used for the injection of adrenalin chloride solution. A chronograph marking either five or fifteen second intervals was placed at the zero blood pressure level. An electro-magnetic signal was used to indicate the point and duration of the injection. In those animals in which the rate of blood flow through the skeletal muscle was also recorded a paraffined glass cannula was placed in the femoral vein all branches to it being ligated except the one coming from a single muscle or group of muscles which we wished to study. The drops of blood leaving the cannula fell on a receiving lever of a tambour connected to another tambour for recording them upon the smoked drum surface.

Adrenalin chloride (P. D. & Co.) (1:100,000 solution) in small volumes was injected into the cannula connected to the femoral vein on the same side as the artery tested. In order to determine whether large doses of epinephrin had the same effect in unanesthetized as it has in anesthetized cats, a 1:10,000 solution of adrenalin chloride was occasionally used after first having tried the more dilute one. Heparin dissolved in warm Ringer's solution was injected intravenously as an anticoagulant in those animals in which the rate of blood flow was studied. It was also used as an anticoagulant in most instances in the mercury manometer system. In the

remaining experiments either sodium citrate or sodium carbonate was used in this system as anticoagulants.

RESULTS. *Effect of epinephrin upon blood pressure.* Our results with dilute solutions of epinephrin upon unanesthetized cats confirmed our earlier findings in decrebrated animals in which the anesthesia had, ½ to 3½ hours prevously, been discontinued. The first injection usually caused

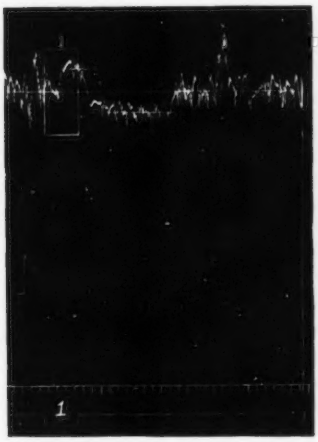

Fig. 1

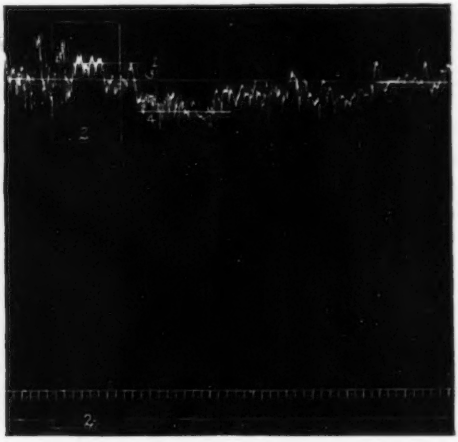

Fig. 2

Fig. 1. Male cat 3.5 kilograms. All operative work except that of cannulating the vessels, had been done under ether anesthesia twenty-four hours before this experiment. Top record blood pressure in millimeters of mercury. Middle time in fifteen seconds and zero blood pressure. Bottom record time of the injection. *1*, time and duration of injection of 0.2 cc. of a 1:100,000 solution of epinephrin; *2*, approximate average of high blood pressure; *3*, normal blood pressure; *4*, approximate average of fall in blood pressure; *X*, animal struggled. Zero points at the end of the record.

Fig. 2. Male cat 2.5 kilograms. Twenty-two hours after operation under ether. Top record blood pressure in millimeters of mercury; below it the time in fifteen seconds and zero blood pressure, bottom record *2*, the time and duration of injection of 0.5 cc. of a 1:100,000 solution of epinephrin. *1*, average of increased blood pressure; *3*, average of normal blood pressure and *4*, average of the fall in blood pressure.

a temporary rise followed by a prolonged fall in blood pressure. The pure depressor response of as marked degree as observed in anesthesized animals was never observed. This difference in action is probably due to the better condition of the vaso-constrictor nerve endings in the unanesthetized animal. That a fall in blood pressure does occur can be seen in figures 1 and 2. In figure 1, 0.2 cc. of a 1:100,000 solution of epinephrin was injected in a 3.5 kilogram cat. A temporary rise in blood pressure of about 12 mm.

of mercury is observed at *2*, followed by a prolonged fall in blood pressure of 10 mm. of mercury as seen at *4*. Line *3* is approximately the mean normal pressure. At *X* the animal struggled causing a sudden increase in blood pressure. Figure 2 is a record taken from another animal weighing 2.5 kilograms. Here *2* indicates the point of the injection and *3* a line indicating the approximate mean normal pressure. Epinephrin 0.5 cc. of a 1:100,000 was injected at *2*. The blood pressure during the injection rose perhaps 8 mm. of mercury at *1*, but fell 16 mm. of mercury immediately thereafter requiring seven minutes to return to the normal pressure level.

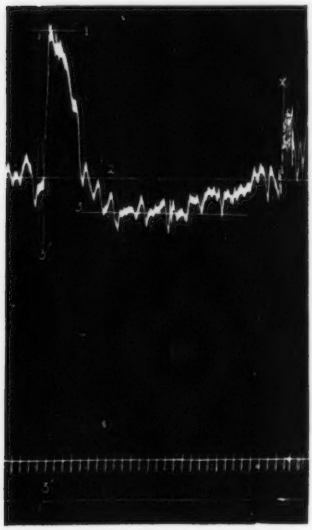

Fig. 3. Same animal as in figure 1. At *3*, 0.2 cc. of a 1:10,000 solution of epinephrin was rapidly injected intravenously. *1*, approximate average of highest blood pressure; *2*, approximate average of normal blood pressure; and *3*, approximate average of fall in blood pressure. *X*, animal struggled.

The unanesthetized cat responds in the same manner to large doses of epinephrin as does the anesthetized one. This is illustrated in figures 3, 6 and 7. In figure 3, at *3*, 0.2 cc. of a 1:10,000 solution of epinephrin was rapidly injected in a 3.5 kilogram cat which had received no ether for twenty-four hours. The blood pressure rose suddenly from 140 to 210 mm. of mercury. In about one minute the blood pressure fell below the normal level to 118 mm. of mercury, returning to normal in approximately five minutes. There was also some indication of vagal stimulation in this record; however this is best seen in figure 6 at *6* in which 0.5 cc. of a 1:10,000 solution of epinephrin was rapidly injected intravenously. Although the blood pressure increased from 147 to 215 mm. of mercury, there was nevertheless a distinct slowing of the heart rate with an increase in the pulse pressure.

The effect of epinephrin upon the blood flow in skeletal muscle. That small doses of epinephrin slowly injected cause dilatation of blood vessels in skeletal muscle can be seen in figure 4. In this figure *O* and *O'* show the relative positions of the two writing points, i.e., blood pressure and that recording the rate of blood flow from the muscles in drops. In this figure as in almost all other records the semimembranosis and semitendinosis muscles were used. At *1*, 0.3 cc. of a 1:100,000, and at *2*, 0.2 cc. of the same concentration of epinephrin were injected intravenously. Although

no appreciable change in blood pressure occurred in either instance, the rate of blood flow nevertheless following the first injection increased from 40 to 80 drops per minute whereas from the second injection it was accelerated to 72 drops per minute.

In another animal in which practically no change in blood pressure occurred after an injection of 0.3 cc. of epinephrin 1:100,000, a change in the

Fig. 4. Male cat, weight 3.8 kilograms. Five hours before this experiment was performed both femoral veins and the left femoral artery had been isolated and freed from their surrounding fascia, both saphenous nerves cut and the spinal cord sectioned in the thoracic region, under deep ether anesthesia. Top record, the blood pressure in the left femoral artery in millimeters of mercury and below it the time interval in fifteen seconds and zero blood pressure. Bottom record the rate of blood flow from the right semimembranosus and semitendinosus muscles in drops, and above it the point and duration of the injection. *O* and *O'* are corresponding positions of the blood pressure writing point and the writing point of the drop recorder. Heparin was used as an anticoagulant both intravenously and in the mercury manometer. *1*, 0.3 cc. of a 1:100,000 solution of adrenalin chloride injected into the left femoral vein. *2*, 0.2 cc. of a 1:100,000 solution of adrenalin chloride similarily injected; *40* and *80* and *40* and *72*, the rates of blood flow before and after the injection of the drug respectively.

rate of blood flow from the muscle from 6 to 26 drops per minute occurred. In this same cat a second injection of epinephrin 0.2 cc. of a 1:100,000 caused a fall in blood pressure of 11 mm. of mercury at the same time the rate of blood flow through the skeletal muscle increased from 15 to 19 drops per minute. In a third animal 0.4 cc. epinephrin 1:100,000 solution was slowly injected intravenously. Without any marked change in blood

pressure the rate of blood flow from the vein leading from the skeletal muscle was increased from 11 to 31 drops per minute.

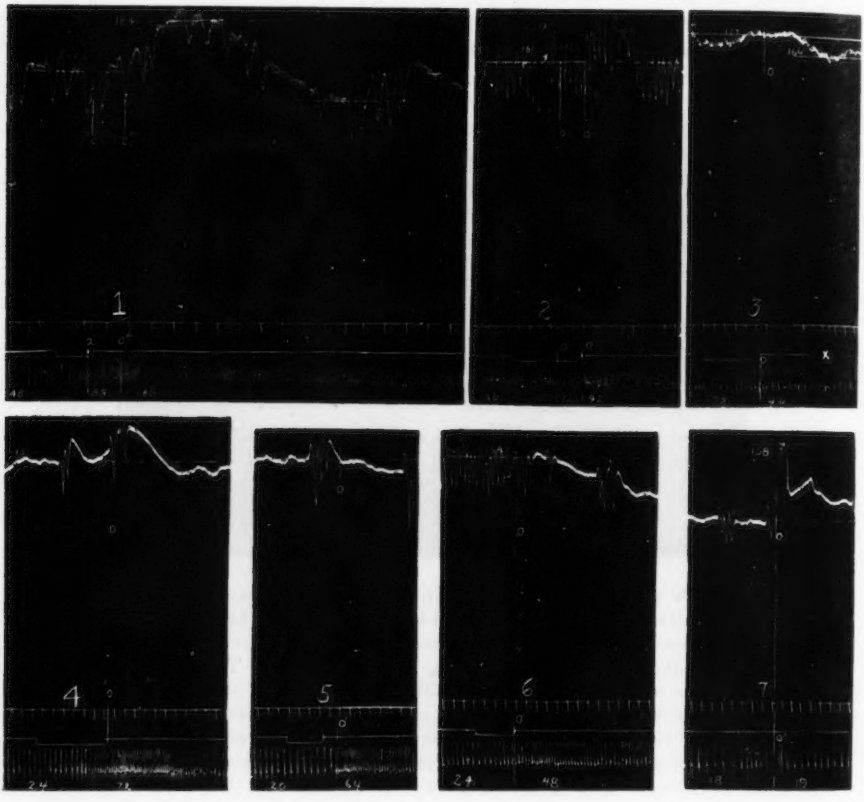

Fig. 5. Male cat 3.8 kilograms. Operative procedure same as in figure 4. No ether for five and one-half hours. Both posterior extremities paralyzed. Heparin injected intravenously as an anticoagulant. In each curve the top record indicates the blood pressure in millimeters of mercury and below it the time interval in five seconds. Bottom record, the drops of blood leaving the leg muscles tested, and above it the duration and time of the injection. *O-O'* and *O''-O'''* are the corresponding positions of the writing points of the mercury manometer and the drop recorder.

Figure 5 shows a series of curves in which epinephrin was injected intravenously in a cat weighing 3.8 kilograms in which the spinal cord had been cut and the administration of ether discontinued for five and one-half hours. In curves *1*, *2* and *4* it will be seen that an increase in the rate of blood flow from the group of muscles is registered before any visible change

in blood pressure occurs. In curves *3*, *5* and *6* an increase in blood flow from the same muscles is recorded in spite of a falling blood pressure. Upon closer analysis of curve *1* it will be noted that the rate of flow increases from 40 to 88 drops per minute before any change in blood pressure is visible. During the interval in which the blood pressure is increased from 153 to 184 mm. of mercury the rate of blood flow from the skeletal muscles is accelerated to 160 drops per minute. However, in the later parts of this curve, although the blood pressure had fallen to 136 mm. of mercury, a decrease of 17 mm., the rate of blood flow from the skeletal muscles continues rapid, being 120 drops per minute. A similar change is seen in curve *2*. In this instance the rate of blood flow increases from 42 to 72 drops per minute without any change in blood pressure but later it increases to 92 drops per minute as the blood pressure increases 16 mm. of mercury. That this increase in blood flow in both curves *1* and *2* cannot be due entirely to changes in the blood pressure can be seen when we compare the rate of flow in these two curves; in the one the rate is quadrupled, in the other doubled, with the change in rate produced by changes in blood pressure as seen in curve *7*. In all instances in which the animal struggled the blood pressure suddenly increased as recorded here. It should also be remembered that the posterior extremities are paralyzed so that they could take no part in the increased activity of the animal and are probably little influenced by the vasomotor center itself. In curve *7* it will be observed that although the blood pressure suddenly increases from 112 to 158 mm. of mercury the increase in rate of blood flow from the skeletal muscles tested is only one drop per minute. In another instance in this same animal the blood pressure increased during struggling from 125 to 168 mm. of mercury and this higher level was maintained for over seventy-five seconds, the increased rate of blood flow through the muscles tested was only seven drops per minute.

More convincing evidence that an active vaso-dilatation of blood vessels in skeletal muscle occurs following the intravenous injection of dilute solutions of epinephrin is seen in curves *3*, *4* and *5* in figure 6. Although here the blood pressure does not increase, the rate of blood flow from the vein of the skeletal muscles increased 16, 44 and 24 drops respectively per minute.

That large doses of epinephrin can produce vaso-constriction as well as vaso-dilatation in skeletal muscles can be noted in figures 6 and 7. In figure 6 at *7*, 1 cc. of a 1:10,000 solution of epinephrin was rapidly injected intravenously in a 3.7 kilogram cat which had received no ether for five hours. No movement of the posterior extremities was possible because of cutting of spinal cord. To avoid the loss of too large a quantity of blood from the animal's circulation through the cannula this injection was made before the effects of the previous one had entirely disappeared as can be seen upon examination of both the blood pressure and rate of flow in curve *6*.

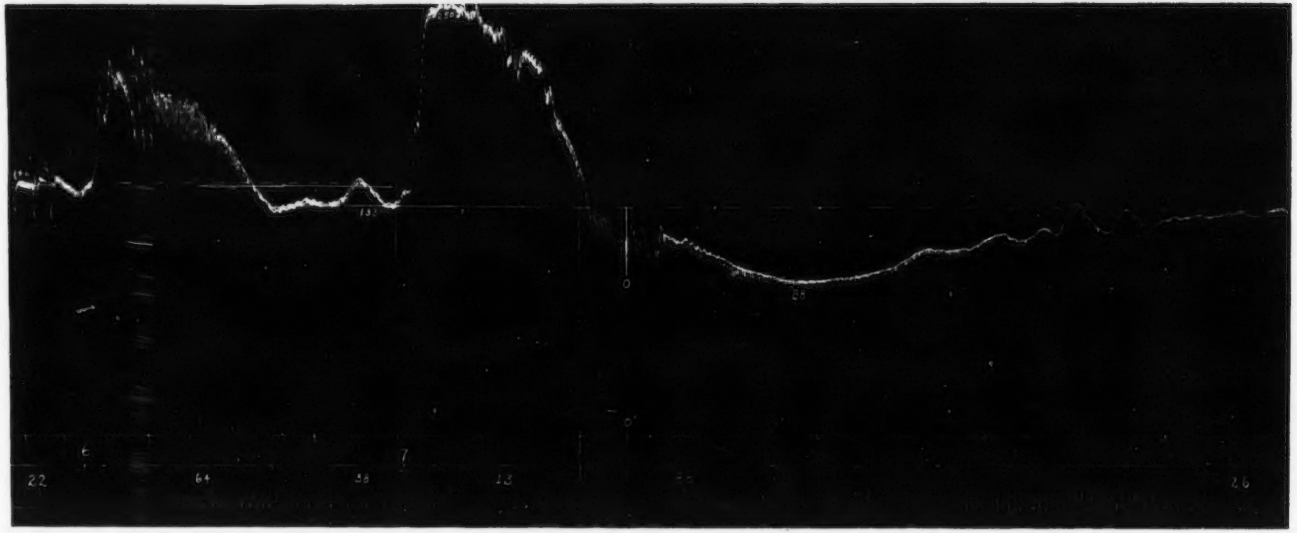

Fig. 6. Male cat weighing 3.7 kilograms. No ether for five hours. Conditions same as in figure 4. *6*, 0.5 cc. of a 1:10,000 solution of adrenalin chloride injected intravenously. *7*, 1 cc. of a 1:10,000 solution of adrenalin chloride injected intravenously.

However, in spite of this fact the blood pressure, as a result of the injection increased from 133 to 250 mm. of mercury with a concomitant change (a decrease of 38 to 13 drops per minute) in blood flow in the muscles tested. The blood pressure did not return immediately to normal but instead dropped 45 mm. below the normal level or to 88 mm. of mercury requiring four minutes to reach the normal level and remain there. Simultaneously

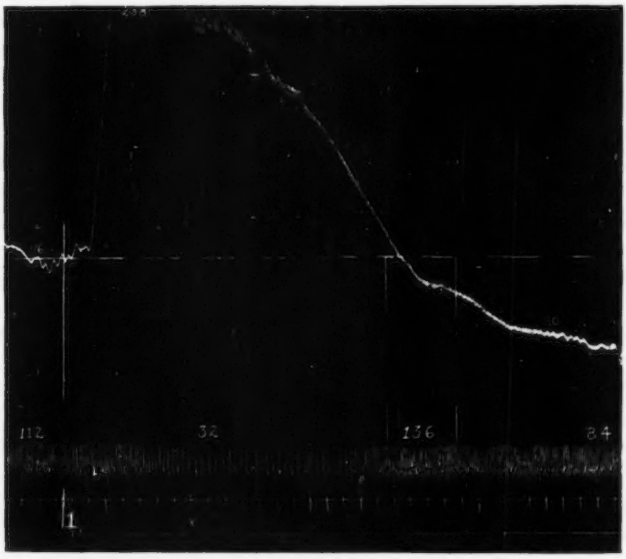

Fig. 7. Male cat weighing 4 kilograms. No ether for five and one-half hours, operative procedure same as in figure 4. Top record, the blood pressure in millimeters of mercury and below it the rate of blood flow in drops from the semimembranosus and semitendinosus muscles. Bottom line, the point of injection and above it the time interval in five seconds and zero blood pressure. Heparin injected intravenously as an anticoagulant. *1*, 1 cc. of a 1:10,000 solution of adrenalin chloride injected intravenously.

with the decrease in blood pressure the rate of blood flow from the skeletal muscles examined increased to 88 drops per minute or 4 times the normal rate of flow when the blood pressure was 146 mm. of mercury.

In figure 7 a 4 kilogram cat was employed which had received no ether for five and one-half hours. Both posterior extremities were paralyzed, through section of the spinal cord. At *1*, 1 cc. of a 1:10,000 solution of epinephrin was injected intravenously. The blood pressure increased from 116 to about 258 (estimation made at the time of the injection the writing point going above the smoked surface of the drum) mm. mercury.

There occurred simultaneously a decrease in the rate of flow of fluid from the muscles tested from 112 to 32 drops per minute. Here again as in figure 6 during the recovery of blood pressure there is an acceleration of the blood flow of 24 drops per minute above the normal rate. This later fell below the normal rate because of excessive loss of blood through the cannula.

Discussion. From the findings presented above it appears that a dilute solution of epinephrin slowly injected is capable of causing vaso-dilatation of the vessels of skeletal muscle with an occasional resultant decrease in blood pressure. In those instances in which the dilatation occurred and in which there was no change in the height of blood pressure we must assume that there occurred simultaneously with the vaso-dilatation a vaso-constriction in other organs of the body. In the anesthetized animal the vessels in which this is the most apt to occur are the cutaneous vessels of the body. The effect of epinephrin upon the blood pressure in unanesthetized as well as in anesthetized animals seems to be dependent upon the balance of action in each case. If the vaso-constrictor endings are more active and more sensitive to epinephrin as appears to be the case in most instances in unanesthetized cats, they overcome the vaso-dilator action of epinephrin in skeletal muscle and rise in blood pressure results. If the effects upon the two systems are equal no change in blood pressure is observed, but if the vaso-dilatation produced in the muscle vessels exceeds the total vaso-constriction produced elsewhere in the body a fall in blood pressure is noted even in unanesthetized animals. The last condition is sometimes observed, but much less frequently than the first. Our results lead us to infer that anesthetics either have a greater depressant effect upon the vaso-constrictor nerve fibers and endings than upon the vaso-dilator ones, or enhance the irritability of the latter fibers and thus favor the depressor responses of epinephrin. The former hypothesis is the more logical. This difference in susceptibility of nerve fibers and endings to anesthetics is in keeping with the results obtained by other observers working on the blood pressure during electrical stimulation of a nerve trunk either centrally or peripherally while changing the surrounding conditions of the nerve (15).

Since epinephrin produces vaso-dilatation in one group of organs and vaso-constriction in another simultaneously we are unable to attribute "hemodynamic effects" in the body to the secretion by the suprarenal glands.

SUMMARY

1. Epinephrin in small doses causes a rise in blood pressure during its intravenous injection in unanesthetized cats but this is followed by a prolonged fall in blood pressure lasting for several minutes. In some animals only a fall is noted.

2. Epinephrin injected intravenously in small doses in unanesthetized cats causes a dilatation of the vessels of skeletal muscles such as that observed in anesthetized animals even though no change in blood pressure may be registered on the kymograph surface.

3 No difference was noticed in the effect of larger doses of epinephrin in unanesthetized animals from that commonly observed in anesthetized animals.

4. Epinephrin in large doses causes vaso-constriction of the blood vessels in skeletal muscles, simultaneously with the marked rise in blood pressure.

5. A fall in blood pressure below the normal level is observed following the increase in blood pressure from large doses of epinephrin. Concomitantly with the fall in blood pressure, there is an increase in the blood flow from skeletal muscles.

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THE EFFECT OF EXTRACTS CONTAINING THE GROWTH PRINCIPLE OF THE ANTERIOR HYPOPHYSIS UPON THE BLOOD CHEMISTRY OF DOGS

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The use of the dog as an experimental animal in the study of acromegaly (1) introduces the possibility of well controlled blood chemistry investigations. The only previous work on blood chemistry in acromegaly has of necessity been confined to human subjects where the difficulty of diagnosis enters in. Even where the diagnosis is certain, one can never be sure that the case is active at the time of investigation. For this reason we have had to start in on our problem with little or no previous idea of what results to expect.

Our first search was for any immediate changes in the blood constituents following the intraperitoneal injection of active extracts of the anterior lobe of the pituitary of the ox.[1] In order to get a lead, preliminary experiments were carried out including blood analysis for calcium, total and inorganic phosphorus, total and preformed creatinine, uric acid, sugar, and total non-protein nitrogen. The dogs used were normal young animals which, after being kept in the laboratory for a month or more on a diet of ground up meat, bread, cod liver oil and yeast showed practically no increase in weight. This was done in order to be reasonably certain that any considerable increase in weight after the injections were started would not be due to normal growth.

In all these experiments whole blood was used in the analyses for all the constituents except calcium, the blood being collected on lithium oxalate cloth. For total and preformed creatinine, uric acid, non-protein nitrogen,

[1] The extracts used in these experiments were made from the fresh anterior lobe of the bovine hypophysis. We wish to state that the extracts used were all modification of the neutralized alkaline ones first described by Evans (4), and Evans and Simpson (5). The actual steps taken have been described in previous communications (2) (3). The criteria for their activity as regards the presence of the growth promoting principle were two:

1. They produced marked increase in weight and definite acromegalic changes in the dogs experimented upon.
2. They were effective in producing abnormal growth in adult female rats.

urea, amino acids and sugar, the blood was precipitated with tungstic acid and analyzed by the Folin colorimetric methods. For total and inorganic phosphorus the blood was precipitated with trichloracetic acid and analyzed by the method of Fiske and Subbarow (6). For serum calcium the method used was one devised by Fiske and Logan (as yet unpublished) and depends upon the precipitation of the dissolved ash of the serum with ammonium oxalate, and ignition of the oxalate precipitate followed by alkalimetric titration of the calcium oxide.

TABLE 1

Dog 160

Weight 16.2 kgm.
Intraperitoneal injection of 40 cc. of freshly prepared extract.
21 hours' fasting before first blood, fasting through experiment.

(Results expressed in milligrams per cent)

REMARKS	PHOSPHORUS WHOLE BLOOD		CREATININE WHOLE BLOOD		URIC ACID WHOLE BLOOD	NON-PROTEIN NITROGEN WHOLE BLOOD	SUGAR WHOLE BLOOD	CALCIUM SERUM
	Total	Inorganic	Preformed	Total				
Just before injection	33.3	4.56	1.39	3.33	1.17	40.5	98.5	10.80
6 hours after injection	33.8	5.15	1.46	3.25	1.20	26.8	99.0	
9 hours after injection	33.0	5.15	1.43	3.20	1.16	26.5	100.0	10.40
13 hours after injection		5.93				25.8		9.00
Maximum change.....	+0.5	+1.37	+0.07	−0.13	+0.03	−14.7	+1.5	−1.80

Dog 161

Weight 17.9 kgm.
Intraperitoneal injection of 40 cc. of a freshly prepared extract.
22 hours' fasting before first blood, fasting through experiment.

Just before injection	33.3	6.40	1.43	3.75	1.23	40.0	91.5	12.20
5 hours after injection	33.8	6.40	1.46	3.80	1.21	33.3	91.0	
8 hours after injection	33.8	6.50	1.42	3.70	1.21	30.0	89.0	11.40
12 hours after injection		7.75				29.8		11.20
Maximum change.....	+0.5	+1.35	+0.03	+0.05	−0.02	−10.2	−2.5	−1.00

It was found by preliminary experiments that no significant changes in the blood constituents were evident until from three to five hours after injection, and that the maximum changes occurred from five to twelve hours after. It is therefore best to follow the blood during this five to twelve hour period. Table 1 gives representative results of this first part of the investigation.

It is apparent from the data given in this table that during the experimental period no significant changes were found in total phosphorus,

TABLE 2

Dog 160

Weight 17.2 kgm.
Intraperitoneal injection of 50 cc. freshly prepared extract.
19½ hours' fasting before first blood, fasting throughout experiment.

(Results expressed in milligrams per cent)

REMARKS	NON-PROTEIN NITROGEN WHOLE BLOOD	UREA WHOLE BLOOD	INORGANIC PHOSPHORUS WHOLE BLOOD	CALCIUM SERUM
Just before injection	37.4		5.52	12.80
4½ hours after injection	37.4		5.94	
6½ hours after injection	37.4		6.15	
7½ hours after injection	35.2		6.15	11.60
14 hours after injection	30.7		6.15	10.20
Maximum changes	−6.7		+0.63	−2.60

Dog 160

Weight 17.0 kgm.
Intraperitoneal injection of 40 cc. freshly prepared extract.
22 hours' fasting before first blood, fasting throughout experiment.

Just before injection	38.1	12.7	6.25	12.80
5 hours after injection	26.4	12.5	6.65	
10 hours after injection	30.3	11.8	6.65	12.60
22 hours after injection	29.6	11.1	6.25	12.80
Maximum changes	−11.7	−1.6	+0.45	−0.20

Dog 161

Weight 19.8 kgm.
Intraperitoneal injection of 55 cc. freshly prepared extract.
21½ hours' fasting before first blood, fasting throughout experiment.

Just before injection	40.0		6.95	13.40
2 hours after injection	35.2		7.15	
4 hours after injection	35.7		7.62	
5½ hours after injection	30.7		7.62	13.40
12½ hours after injection	29.3		7.62	13.20
Maximum changes	−10.7		+0.67	−0.20

Dog 161

Weight 21.3 kgm.
Intraperitoneal injection of 35 cc. freshly prepared extract.
22 hours' fasting before first blood, fasting throughout experiment.

Just before injection	40.0	15.8	8.00	14.60
5 hours after injection	34.3	15.2	8.40	
10 hours after injection	30.3	14.0	8.00	12.60
22 hours after injection	30.0	12.1	7.42	13.60
Maximum changes	−10.0	−3.7	−0.68	−2.00

preformed or total creatinine, uric acid or sugar. The analysis of the blood for these constituents was therefore discontinued at this point.

The most striking change observed was the marked drop in non-protein nitrogen following the injection. It was decided, therefore, to concentrate our attention upon the non-protein nitrogen constituents of the blood and leave the following up of any possible changes in the other substances to another time.

The observation of a slight drop in serum calcium coincident with a slight rise in inorganic phosphorus as shown in table 1 seemed to us rather suggestive, however, and for this reason we include table 2 which shows the result of a few further experiments upon this particular phase.

As is seen, these further experiments were rather disappointing. In the first three the rise in inorganic phosphorus is not great enough to be significant and in the last one there is an apparent fall in inorganic phosphorus. The drop in calcium also does not always occur after injection, and in these few experiments there appears to be no relation between the two.

It is possible that more work upon calcium and phosphorus in the blood after injection of anterior lobe extract might be enlightening, but we decided to confine ourselves in the rest of this investigation to the non-protein nitrogen constituents, in which, as is shown in both tables 1 and 2, the change is rather striking. We did, however, carry out some occasional calcium determinations and these will be included among the other data which are to be presented.

Control experiments. In the attempt to convince ourselves that any changes observed in the blood constituents after injection were due to the active growth principle of the extracts used, a number of control experiments were carried out.

In the first place the blood was followed at intervals during twelve to forty-four hour fasting periods (table 3).

As a result of these and other experiments it was concluded that it was not safe to consider that the fasting level of non-protein nitrogen constituents had been reached until twenty hours after the food had been removed, but that after this time and up to forty-four hours at least there were practically no fluctuations. Morgulis (7), (8) has found that very long periods of fasting (weeks) cause an increase in blood non-protein nitrogenous constituents. We need not be concerned in these experiments, however, with having the results affected by fasting, provided the experiment is not started until a twenty hour fast has been completed.

It is possible that the extracts of the anterior hypophysis may contain slight amounts of oxytocin and vasopressin. Therefore, to make sure that this would not cause a drop in blood non-protein nitrogen constituents, the experiment reported in table 4 was carried out.

The amount of pituitrin given was a large normal human dose, a great

deal more than could be present as an impurity in the anterior hypophyseal extracts used. It is evident that the changes observed could not be ascribed to pituitrin.

TABLE 3

Control experiments on fasting animals

Dog 158

No. 1. Weight 15.3 kgm.
Blood followed during fasting period.
No injection.

(Results expressed in milligrams per cent)

REMARKS	NON-PROTEIN NITROGEN WHOLE BLOOD	UREA WHOLE BLOOD	INORGANIC PHOSPHORUS WHOLE BLOOD	CALCIUM SERUM
After 12 hour's fasting	84.4		4.70	
After 15 hour's fasting	57.6		5.63	11.80
After 20 hour's fasting	34.5		5.35	
After 25¾ hour's fasting	34.3		4.91	
After 35½ hour's fasting	34.0			
Maximum changes after fasting level was reached.....	−0.5		−0.44	

Dog 158

No. 2. Weight 15.0 kgm.
Blood followed during fasting period.
No injection.

After 22 hour's fasting	36.6	16.7	5.45	11.80
After 27 hour's fasting	38.5	16.4	5.62	
After 32 hour's fasting	37.4	16.5	5.33	
After 44 hour's fasting	37.0	16.7	5.10	11.60
Maximum changes.....	−1.5	+0.3	−0.52	−0.20

Dog 159

No. 3. Weight 10.4 kgm.
Blood followed during fasting period.
No injection.

After 22 hour's fasting	32.4	14.0	4.36	12.50
After 27 hour's fasting	33.0	13.0	4.45	
After 32 hour's fasting	32.1	13.7	4.45	
After 44 hour's fasting	32.4	13.5	4.10	12.00
Maximum changes.....	−0.9	−1.0	−0.35	−0.50

The growth principle of the anterior hypophysis is destroyed by boiling. A number of experiments were done in which extracts were inactivated by boiling for six minutes and then injected into the dogs intraperitoneally. The results are given in table 5.

The results of experiment 1 of this series are not very satisfactory, inasmuch as the active extract was followed by only a slightly greater drop in non-protein nitrogen than was the inactive. We have had two other such cases in which dogs that had been showing time after time a marked drop in non-protein nitrogen constituents after the injection of active extract suddenly gave little or no response. No explanation has been found for this phenomenon. In the other three experiments reported in table 5,

TABLE 4

Dog 161

Weight 27.6 kgm. (Dog had been receiving daily injections of anterior hypophysis extracts for 38 days and during that time gained 7.8 kilograms in weight.)

1st injection—1 cc. of pituitrin S (normal human dose).

2nd injection—50 cc. of Parke, Davis & Company's* extract of the anterior hypophysis of the ox. Extract Rx093985A.

(Results expressed in milligrams per cent)

HOURS FASTING	REMARKS	NON-PROTEIN NITROGEN WHOLE BLOOD	UREA WHOLE BLOOD	AMINO ACIDS WHOLE BLOOD
hours				
20	5½ hours before 1st injection	39.0	19.4	9.32
22½	2½ hours before 1st injection	39.0	19.4	9.21
25½	Just before the injection of 1 cc. of pituitrin S	38.6	19.0	9.44
30½	5 hours after injection of pituitrin	39.2	19.6	9.10
42	17 hours after injection of pituitrin. Just before injection of 50 cc. of active anterior lobe extract	38.4	19.0	9.44
47	5 hours after injection of anterior lobe extract	28.4	15.6	8.00
49	7 hours after injection of anterior lobe extract	29.9		
Maximum changes after pituitrin		+0.6	+0.6	−0.34
Maximum changes after anterior lobe extract		−10.0	−3.40	−1.44

* Parke, Davis & Co. have coöperated with us in preparing some of the extracts according to the specifications given by H. M. Teel, Science, 1929, lxix, 405.

however, the results seem to show pretty conclusively that whatever causes the disappearance of non-protein nitrogen from the blood is destroyed by the boiling of the extract. The results of experiments 3 and 4 of this series are particularly conclusive. The inactivated extract was given in these two cases after the non-protein nitrogen had started to rise again following the drop caused by the active extract. As is seen, this rise continues even after the inactive extract is injected. In table 8 three experiments are reported in which second injections of *active* extract were

TABLE 5

Control experiments using inactivated extract

Dog 160

No. 1. Weight 18.7 kgm. Had been receiving daily injections of active extract for 22 days, with gain in weight of 2.5 kgm.

1st injection—Intraperitoneal injection of 35 cc. of a fresh extract which had been inactivated by boiling.

2nd injection—Intraperitoneal injection of 25 cc. of a fresh active extract (modification of Evans).

Dog had fasted 20 hours before and fasted throughout experiment.

(Results expressed in milligrams per cent)

REMARKS	NON-PROTEIN NITROGEN WHOLE BLOOD	UREA WHOLE BLOOD	AMINO ACIDS WHOLE BLOOD
2 hours before 1st injection	33.3	15.2	9.30
Just before injection of inactivated extract	33.4	12.8	8.85
5 hours after injection of inactivated extract	31.3	12.8	8.20
10 hours after injection of inactivated extract	32.8	12.0	9.15
22 hours after injection of inactivated extract			
Just before injection of active extract	35.0	13.0	9.50
5 hours after injection of active extract	32.5		
Maximum changes after inactive extract..	−1.1	−0.8	−0.65
Maximum changes after active extract....	−2.5		

Dog 161

No. 2. Weight 21.8 kgm. Had been receiving daily injections of active extract for 22 days, with gain in weight of 3.9 kgm.

Injections—same as in above experiment.

Dog had fasted 20 hours before and fasted throughout experiment.

2 hours before 1st injection	29.5	12.3	8.75
Just before injection of inactivated extract		12.1	
5 hours after injection of inactivated extract	28.8	11.2	8.30
10 hours after injection of inactivated extract	30.0	11.0	8.75
22 hours after injection of inactivated extract			
Just before injection of active extract	31.2	12.5	8.20
5 hours after injection of active extract	24.0		
Maximum changes after inactivated extract.	−0.7	−1.1	−0.40
Maximum changes after active extract....	−7.2		

TABLE 5—*Concluded*

Dog 159

No. 3. Weight 11.8 kgm. This dog had been receiving daily injections of active extract with gain in weight of 1.4 kgm.

1st injection—Intraperitoneal injection of 50 cc. of fresh active extract.

2nd injection—Intraperitoneal injection of 50 cc. of same extract inactivated by boiling.

Dog had fasted 23 hours before and fasted throughout experiment.

REMARKS	NON-PROTEIN NITROGEN WHOLE BLOOD	UREA WHOLE BLOOD	AMINO ACIDS WHOLE BLOOD	CALCIUM SERUM
2 hours before 1st injection	38.0	14.7	9.00	
Just before injection of active extract	39.3	14.5	9.20	
4 hours after injection of active extract	29.2	13.3	6.90	
5 hours after injection of active extract	29.6	12.5	7.10	
6 hours after injection of active extract	30.0	12.5	7.00	
8½ hours after injection of active extract	27.0	10.6	7.20	
22½ hours after injection of active extract	25.7	9.6	7.20	
29½ hours after injection of active extract	25.8			
45½ hours after injection of active extract				
Just before injection of inactivated extract	27.3			
4 hours after injection of inactivated extract	28.3			
7 hours after injection of inactivated extract	32.0			
Maximum changes after active extract	−13.6	−5.1	−2.20	
Maximum changes after inactive extract	+4.7			

Dog 169

No. 4. Weight 13.2 kgm. Never before injected.

1st injection—Intraperitoneal injection of 50 cc. of Parke, Davis & Co. active extract, Rx093995.

2nd injection—Intraperitoneal injection of 50 cc. of same extract inactivated by boiling.

Dog had fasted 22 hours before and fasted throughout experiment.

4 hours before 1st injection	36.3	15.6	5.85	
2 hours before 1st injection	36.8			
Just before injection of active extract	36.3	15.8	6.05	13.20
4 hours after injection of active extract	28.5			
7½ hours after injection of active extract	25.3	12.8	6.10	11.60
24¾ hours after injection of active extract	33.3			
26¾ hours after injection of active extract				
Just before injection of inactivated extract	34.3	14.0	6.60	
6½ hours after injection of inactivated extract	35.2			
18 hours after injection of inactivated extract	36.8	15.0	6.70	
Maximum changes after active extract	−11.5	−3.0	+0.55	−1.60
Maximum changes after inactive extract	+2.5	+1.0	+0.10	

given. The comparison of these later results with the control experiments given here furnishes fairly strong evidence in favor of our conclusion that the drop in non-protein nitrogen and urea is caused by a heat labile constituent of the extract, probably the growth producing principle.

It was thought well to ascertain whether or not an unheated body protein which contained no growth principle would have any effect upon the non-protein nitrogen constituents of the blood. For this purpose a neutral sodium acetate extract of bovine serum protein was made up, containing the same percentage of nitrogen (i.e., about 1 gram per 100 cc.) as do the preparations of anterior hypophysis extract. This preparation is extraordinarily similar in appearance to the extracts which contain the active

TABLE 6

Control experiment using serum protein

Dog 170

Injected once before with active anterior lobe extract and showed a drop in non-protein nitrogen of 10.2 mgm. per 100 cc. whole blood (see table 9, no. 1).

Injected this time intraperitoneally with 50 cc. of a neutral sodium acetate extract of serum protein.

Dog had fasted for 20 hours and fasted throughout experiment.

(Results expressed in milligrams per cent)

REMARKS	NON-PROTEIN NITROGEN WHOLE BLOOD	UREA WHOLE BLOOD	AMINO ACIDS WHOLE BLOOD
1½ hours before injection	34.2	17.4	9.55
Just before injection of serum protein extract	33.8	17.6	9.65
5 hours after injection of serum protein extract	34.5	17.8	9.80
6½ hours after injection of serum protein extract	33.5	17.8	9.65
Maximum changes after injection.....	−0.70	+0.4	+0.10

principle. As is shown in table 6, it has no effect upon the non-protein nitrogen, urea, or amino acids of the blood.

The results of these control experiments seem to indicate that any drop in non-protein nitrogen and urea observed after the injection of an extract of the anterior lobe of the pituitary is probably caused by the active growth principle of this gland. (Further work was done in the course of this investigation with especial reference to changes in amino acids, and these results will be reported and discussed below.)

Experiments in which the non-protein nitrogen constituents of the blood were followed after the injection of active extracts of the anterior hypophysis. The experiments already reported show that the injection into a dog of a growth promoting extract of the anterior hypophysis is followed by a dis-

appearance from the blood of an appreciable amount of the non-protein nitrogen constituents. Part of this loss is accounted for by a drop in the urea and amino acids. Quantitatively the results have not been uniform.

TABLE 7

Showing the response to injections of active extracts

Dog 160

No. 1. Weight 17.2 kgm. This dog had been receiving daily injections for 21 days with a gain in weight of 1 kgm.

Injection—Intraperitoneal of 50 cc. of a fresh active extract.

Dog fasted 20 hours before and fasted throughout experiment.

(Results expressed in milligrams per cent)

REMARKS	NON-PROTEIN NITROGEN WHOLE BLOOD	UREA WHOLE BLOOD	AMINO ACIDS WHOLE BLOOD	CALCIUM SERUM
Just before injection of active extract	39.3	12.6	9.65	
5½ hours after injection of active extract	28.5	10.7	7.75	
Drop after injection	−10.8	−1.9	−1.90	

Dog 161

No. 2. Weight 21.8 kgm. Had been receiving daily injections for 21 days with a gain in weight of 3.9 kgm.

Injection—Same as above.

Dog fasted 20 hours before and fasted throughout experiment.

Just before injection of active extract	38.7	15.6	10.00	
5½ hours after injection of active extract	30.7	14.0	9.05	
Drop after injection	−8.0	−1.6	−0.95	

Dog 158

No. 3. Weight 15.8 kgm. Had never been injected before.

Injection—Intraperitoneal of Parke, Davis & Co. Rx093985A.

Dog fasted 19 hours before and fasted throughout experiment.

4 hours before injection	33.3	13.0	8.00	
2 hours before injection	34.2	11.8		
Just before injection of Rx093985A	32.8	12.0	8.00	12.00
3 hours after injection of Rx093985A	29.2	11.4	7.71	
6½ hours after injection of Rx093985A	27.2	9.30	7.52	9.40
19 hours after injection of Rx093985A	27.2		7.20	
Maximum drop after injection	−5.6	−2.70	−0.80	−2.60

Not only do different dogs vary both in the time and the degree of their responses, but the same dog may vary at different times. These facts are further demonstrated by the results given in tables 7 and 8.

TABLE 8

Showing the response to repeated injections of active extracts

Dog 158

No. 1. Weight 20.8 kgm. Had been receiving daily injections for 21 days with a gain in weight of 5 kgm.

1st injection—Intraperitoneal of 50 cc. of Parke, Davis & Co. Rx093995.

2nd injection—Intraperitoneal of 50 cc. of Parke, Davis & Co. Rx093983A.

Dog had fasted 20 hours before and fasted throughout experiment.

(Results expressed in milligram per cent)

REMARKS	NON-PROTEIN NITROGEN WHOLE BLOOD	UREA WHOLE BLOOD	AMINO ACIDS WHOLE BLOOD	CALCIUM SERUM
2 hours before injection	35.2	16.1	9.35	
Just before 1st injection Rx093995	35.0	16.3	9.25	
5 hours after 1st injection Rx093995	28.2	12.7	8.25	
7 hours after 1st injection Rx093995	27.8	12.2	7.70	
21 hours after 1st injection Rx093995	31.8	14.8	8.60	
26 hours after 1st injection Rx093995				
Just before 2nd injection Rx093983A	35.2			
5½ hours after 2nd injection Rx093983A	30.5			
Maximum drop after 1st injection.....	−7.2	−4.1	−1.45	
Maximum drop after 2nd injection.....	−4.7			

Dog 168

No. 2. Weight 12.2 kgm. Never before injected.

1st injection—Intraperitoneal of 30 cc. of fresh extract.

2nd injection—Intraperitoneal of 55 cc. of fresh extract.

4 hours before injection	34.2	14.6	6.80	
2 hours before injection	34.5			
Just before 1st injection of active extract	34.5	14.0	6.70	13.20
4 hours after 1st injection of active extract	30.0			
7½ hours after 1st injection of active extract	31.0	12.8	6.77	11.20
24¾ hours after 1st injection of active extract	35.2			
26½ hours after 1st injection of active extract				
Just before 2nd injection of active extract	37.5	14.2	7.00	
6½ hours after 2nd injection of active extract	37.0			
18 hours after 2nd injection of active extract	31.5	12.7	5.87	
Maximum drop after 1st injection.....	−4.5	−1.8		−2.00
Maximum drop after 2nd injection.....	−6.0	−1.5	−1.13	

TABLE 8—*Concluded*

Dog 159

No. 3. Weight 11.2 kgm. Never before injected.
1st injection—Intraperitoneal of 50 cc. of Parke, Davis & Co. Rx093985A.
2nd injection.—The same.
Dog had fasted for 22 hours and fasted throughout experiment.

REMARKS	NON-PROTEIN NITROGEN WHOLE BLOOD	UREA WHOLE BLOOD	AMINO ACIDS WHOLE BLOOD	CALCIUM SERUM
2 hours before 1st injection	38.1	16.8	8.00	
Just before 1st injection Rx093985A	38.0	16.6	7.90	12.00
3 hours after 1st injection Rx093985A	31.8	13.6	7.00	
5 hours after 1st injection Rx093985A	25.0	9.22	6.18	
8 hours after 1st injection Rx093985A	26.0	11.60	6.75	
10 hours after 1st injection Rx093985A	25.7	9.40	7.10	10.60
12½ hours after 1st injection Rx093985A	25.5	9.22	7.20	
22½ hours after 1st injection Rx093985A	26.7	10.1	7.30	
26½ hours after 1st injection Rx093985A	30.8	10.8	7.40	
28½ hours after 1st injection Rx093985A				
Just before 2nd injection of Rx093985A	31.5	11.4	7.80	
5 hours after 2nd injection of Rx093985A	24.5	8.70	6.37	
6 hours after 2nd injection of Rx093985A	24.3	8.90	6.37	
Maximum drop after 1st injection.....	−13.1	−7.60	−1.82	−1.40
Maximum drop after 2nd injection.....	−7.20	−2.70	−1.43	

The examination of the tables will show that it is impossible to draw any conclusions concerning the quantitative effect of the anterior lobe extract upon the non-protein nitrogen constituents of the blood. (It must be realized that in taking bloods at arbitrary intervals during the experimental period we may quite possibly have missed the maximum changes in some cases.) A given amount of extract cannot be said to cause a given percentage drop in a given time. Even the same dog appears to differ in his response at different times. It is extremely interesting to note, however, that in one rather important respect, the quantitative relations appear to be quite consistent. That is, it may be said that in general the drop in urea is somewhere around half that in the non-protein nitrogen, while from 10 to 20 per cent of the drop in total non-protein nitrogen constituents is often accounted for by the drop in amino acids. This is in keeping with the percentage composition of the blood and would seem to indicate that the anterior lobe extract causes a disappearance from the blood of urea, amino acids, and also of those non-protein nitrogen constituents of the blood which must be grouped under the head of "undetermined nitrogen." If it had been found that all of the drop in non-protein nitrogen could be accounted for by the drop in urea, we would have been forced to conclude

that the effect was merely one of increased excretion. It would seem reasonable that a growth promoting hormone should cause a metabolic mobilization of the nitrogenous constituents of the blood for the purposes of building up new protoplasm. We have not been able to get any really conclusive evidence that some of the disappearance of non-protein nitrogen constituents of the blood might not be attributed to increased excretion. But the fact that amino acids and "undetermined" non-protein nitrogen constituents also disappear, and in amounts which are in keeping with the percentage composition of the blood, would seem to indicate that the effect is at least in part endogenous.

Attempts to discover whether or not increased excretion of nitrogen accompanies its disappearance from the blood of an injected dog. Having demonstrated that a disappearance of appreciable amounts of the nitrogenous constituents of the blood follows the injection of anterior lobe extracts, our next concern was to get some indication as to where these constituents went. It might at first seem reasonable to analyze the urine in order to ascertain whether or not increased excretion occurs. In short experiments of this sort, however, this is entirely impracticable for a number of reasons. In the first place, we have only a very small amount of nitrogen to account for in comparison with the nitrogenous composition of the urine, an amount which would fall within the limits of experimental error. The first source of experimental error in analysing the urine of a dog lies in the difficulty of collecting every drop of the urine which is excreted during the experimental period. Even if this error is decreased by keeping the dog on the table for the eight or more hours (a difficult thing to do) and washing out the bladder after each catheterization, the results are still not dependable to the point of accuracy which would be required in order to account for the small amounts of nitrogen which we are attempting to trace. Only one experiment of this kind was necessary to convince us of the impossibility of *directly* proving whether or not increased excretion occurs. This is reported in table 9.

From the results given here it is seen that large fluctuations appear in the hourly excretion of nitrogen and urea. In this particular experiment a cutting down on urine formation appeared to occur during the first two hours after injection. No conclusions can evidently be drawn from the hourly excretion. The blood volume of this dog is probably about 2000 cc. We therefore have 180± mgm. of nitrogen and 64± mgm. of urea to account for in the period following the injection. Considering the total excretion during this period, the impossibility of tracing such small amounts is evident. It might be noted that during the second to sixth hour after injection there is a greater excretion of urea in comparison to the excretion of total nitrogen than occurred during the other periods. However, this actually represents a difference in colorimeter readings of only one milli-

meter. Unless greater differences than these are observed no conclusions can be drawn. On the whole, it was decided that the limits of accuracy of urine analysis did not warrant spending more time upon this particular type of experiment.

It was thought that if we could cause a cutting down of urine formation during the experimental period and still observe a drop in the non-protein nitrogen constituents of the blood after injection, we would have some

TABLE 9

Urine and blood analysis after injection of 50 cc. of fresh extract
Dog 168

Weight 12.2 kgm.
Dog had fasted for 20 hours and fasted throughout experiment.

REMARKS	URINE				WHOLE BLOOD		
	Time	Volume	Total nitrogen	Urea	N. P. N.	Urea	Amino acids
			mgm.	*mgm.*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
2 hours before injection	8:40 a.m.	a.m. urine discarded			39.0	16.1	9.55
Just before injection	8:40 to 10:40 a.m.	20 cc. ± made up to 100 cc.	575	510	38.6	16.2	9.65
2 hours after injection	10:40 to 12:40 p.m.	10 cc. ± made up to 100 cc.	200	157	39.0	16.4	9.65
3 hours after injection					35.6	15.4	9.25
4 hours after injection	12:40 to 2:45 p.m.	30 cc. ± made up to 100 cc.	662	666	34.2	14.6	9.25
6 hours after injection	2:45 to 4:40 p.m.	20 cc. ± made up to 100 cc.	449	465	32.0	14.4	9.10
8 hours after injection	4:40 to 6:40 p.m.	20 cc. ± made up to 100 cc.	575	508	30.0	13.0	9.00
Total excretion in the urine 8 hours after injection	10:40 to 6:40 p.m.	80 cc. ± made up to 400 cc.	1,886	1,796			
Maximum drop in blood constituents after injection					−9.0	−3.2	−0.65

evidence against increased excretion of nitrogenous substances. Poisoning of the kidney or tying off of the ureters would, of course, result in so great a rise in blood nitrogen and urea as to obscure any drop caused by the extract. It was thought, however, that some indication might be gained by depriving the dog of water during the experimental period, and so cutting down on the formation of urine. Table 10 shows the results of two such experiments.

In the first experiment of this series a blood sample was taken on the morning after the injection, that is, twenty-one hours after injection and

TABLE 10

Showing the response to injection when dog is deprived of water

Dog 158

No. 1. Weight 24.4 kgm.
Injections—45 cc. of fresh anterior lobe extract.
Dog had fasted 24 hours before and throughout experiment.
No water for 12 hours before and throughout experiment.

REMARKS	HAEMATOCRIT PER CENT CORPUSCLES	NON-PROTEIN NITROGEN			UREA		
		Whole blood	Plasma	Corpuscles calculated	Whole blood	Plasma	Corpuscles calculated
		mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
1¼ hours before injection	33.5	38.0	32.0	50.2	18.5	16.9	19.0
Just before injection	33.5	39.2	33.4	51.2	18.0	17.1	19.9
4 hours after injection	33.0	38.6	32.5	51.6			
5 hours after injection	32.5	35.2	29.4	47.5	16.2	14.8	18.7
6 hours after injection	34.0	36.9	30.6	49.5	16.4	14.6	19.7
21 hours after injection. Urine excreted during these 22¼ hrs. = 340 cc.	34.0	45.2	40.0	55.6	23.0	22.5	24.4
Maximum drop after injection		−4.0	−4.0	−3.7	−1.8	−2.3	−1.2
Total rise during 22¼ hours		+7.2	+8.0	+5.4	+5.1	+5.6	+5.4

Dog 159

No. 2. Weight 15 kgm.
Injection—50 cc. of fresh anterior lobe extract.
Dog had fasted 24 hours before and throughout experiment.
No water for 3 hours before injection and throughout experiment.

2 hours before injection	30.0	35.6	30.6	47.3	18.7	19.8	16.3
Just before injection	30.0	35.7	30.6	47.5	18.5	19.7	16.0
4 hours after injection	30.0	33.8	28.9	46.2	18.5	18.2	19.3
5 hours after injection	30.0	33.6	30.6	40.6	18.2	17.3	20.2
6 hours after injection	30.0	30.0	29.3	31.6	18.2	16.9	21.3
8 hours after injection. Urine excreted during 24 hr. period in which no water taken = 230 cc.	31.0	30.6	29.6	32.9	18.7	16.9	22.5
Maximum drop after injection		−5.6	−1.3	−15.7	−0.5	−2.9	+5.0

when the dog had been without water for thirty-six hours. Apparently depriving the dog of water and so cutting down on urine formation results in a rise in the nitrogenous constituents of the blood. In spite of this

TABLE II

Whole blood, plasma and cells (by calculation) after the injection of anterior lobe extract

Dog 170

No. 1. Never before injected.
Injection—Intraperitoneal of 50 cc. of fresh active extract.
Dog had fasted for 20 hours and fasted throughout experiment.

REMARKS	HAEMATOCRIT	NON-PROTEIN NITROGEN			UREA			AMINO ACIDS		
		Whole blood	Plasma	Corpuscles calculated	Whole blood	Plasma	Corpuscles calculated	Whole blood	Plasma	Corpuscles calculated
		mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
2 hours before injection	46 5	44 5	36 3	54 2	23 0	20 7	25 6	10 9	7 30	15 0
Just before injection	46 5	45 0	36 3	55 0	22 5	20 7	24 6	11 0	7 00	15 6
5½ hours after injection	45 0	34 8	26 0	45 5	17 1	13 8	21 2			
6½ hours after injection	44 5	35 2	28 5	44 0	17 4	14 6	20 5	11 0	5 20	18 3
21½ hours after injection	46 5	38 0	29 6	48 5	16 9	13 6	19 6	10 3	5 35	16 0
Maximum changes after injection.....		−10 2	−10 3	−9 5	−5 6	−7 1	−5 0	−0 6	−2 10	+3 30

Dog 171

No. 2. Weight 13.4 kgm. Never before injected.
Injection—Intravenous of 50 cc. of fresh active extract.
Dog had fasted for 20 hours and fasted throughout experiment.

1½ hours before injection		40 0			18 8					
Just before injection	42 5	39 2	35 4	44 6	19 0	18 3	20 0	9 65	5 51	15 3
1 hour after injection		38 0								
2 hours after injection	42 0	36 2	33 0	41 1	17 0	17 4	16 7	9 52	6 10	14 2
3 hours after injection		32 6			15 4					
4 hours after injection	40 0	31 5	28 2	36 6	15 0	13 9	16 7	9 65	3 12	19 5
5 hours after injection	39 0	35 3	30 3	43 2	17 3	15 4	20 3	8 02	5 06	12 7
Maximum changes after injection.....		−7 7	−7 2	−8 0	−4 0	−4 4	−3 3	−1 63	−2 39	+4 2

Dog 168

No. 3. Weight 12.2 kgm. Had received a few previous injections.
Injection—Intravenous of 50 cc. of fresh active extract.
Dog had fasted for 20 hours and fasted throughout experiment.

2 hours before injection	46 0	39 0	34 6	44 1	16 1	20 7	10 7	9 55	6 85	12 7
Just before injection	47 0	38 6	34 0	43 7	16 2	20 7	10 6	9 65	7 00	12 7
2 hours after injection	44 0	39 0			16 4			9 65		
3 hours after injection		35 6			15 4			9 25		
4 hours after injection	41 0	34 2	31 5	38 0	14 6	17 9	12 0	9 25	6 00	13 9
6 hours after injection	40 0	32 0	29 0	36 4	14 4	16 0	11 9	9 10	5 78	14 1
8 hours after injection	38 0	30 0	26 8	35 0	13 0	14 4	10 8	9 00	5 52	14 7
Maximum changes after injection.....		−9 0	−7 8	−9 1	−3 2	−6 3	+1 4	−0 65	−1 48	+2 0

Male French Bull

No. 4. Never before injected.
Injection—Intraperitoneal of 50 cc. of fresh active extract.
Dog had fasted 24 hours and fasted throughout experiment.

2 hours before injection	46 0	30 0			12 1	12 3	11 8	10 3	7 77	13 3
Just before injection	46 0	29 8			12 1	12 3	11 8	10 3	7 77	13 3
3 hours after injection	45 0	29 3			11 2	11 6	10 8	8 9	5 52	13 0
4 hours after injection	44 0	27 0			10 4	10 9	9 8	8 65	6 35	11 6
5 hours after injection	43 5	24 0			10 2	10 3	40 1	7 9	5 16	11 5
6 hours after injection	42 5	23 5			9 3	7 9	11 2	9 00	4 95	17 3
Maximum changes after injection.....		−6 5			−2 8	−4 4	−2 0	−2 40	−2 82	+4 9

TABLE 12

Whole blood, plasma and cells (by direct analysis) during control periods and after injection of anterior lobe extract

Dog 174

No. 1. Never injected.
Control experiment without injection.

REMARKS	HAEMATOCRIT	NON-PROTEIN NITROGEN				UREA				AMINO ACIDS			
		Whole blood	Plasma	Cells by analysis	Cells calculated	Whole blood	Plasma	Cells by analysis	Cells calculated	Whole blood	Plasma	Cells by analysis	Cells calculated
		mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
After 20 hour's fast	40.5	29.0	25.0	31.0	35.0					9.10	8.25	14.9	10.4
After 21 hour's fast	38.5	29.0	25.0	31.0	35.4					8.75	6.65	14.7	12.1
After 22 hour's fast	35.0	28.4	24.6	31.4	35.3					9.35	5.33	15.4	16.7
After 23 hour's fast	33.5	28.2	24.4	31.4	35.6					8.75	6.35	15.4	13.6
After 39½ hour's fast	36.5	28.6	24.8	31.5	35.4					8.75	5.37	15.6	14.6
Maximum changes during control period		−0.8	−0.6	+0.5	+0.6					−0.60	−2.92	−0.9	+6.3

Dog 172

No. 2. Weight 22.5 kgm. Never before injected.
4 hour control experiment followed by intraperitoneal injection of 50 cc. fresh extract.
Dog had fasted 20 hours before and fasted throughout experiment.

After 20 hour's fast	46.0	43.5	42.1		45.4	20.8	22.0	21.0	19.7	10.9	7.20	15.7	15.3
After 21 hour's fast	44.0	44.5	42.6		47.5	21.3	22.2	20.7	20.3	10.9	7.30	15.8	15.7
After 22 hour's fast	42.0	44.0	43.2		45.2	21.1		20.7		11.2	6.75	15.6	17.3
After 23 hour's fast										11.2	6.50	15.7	17.6
Just before injection	41.0	43.5	42.0		46.0	20.6	22.2	20.6	18.7				
3 hours after injection	43.5	39.5	37.0		43.0	20.4	22.2	20.7	18.2	10.6	7.75	15.9	14.3
4 hours after injection	42.0	36.4	35.6		37.7					10.2	8.00	15.0	13.2
5 hours after injection	40.0	30.7	30.0		32.0	20.4	22.2	21.0	17.8	9.4	8.15	14.1	11.2
22½ hours after injection	38.5	33.6	34.7		33.0	18.1	17.3	20.7	19.4	10.1	7.30	15.7	14.5
Maximum changes during control period		+1.0	+1.1		+2.1	−0.7	+0.2	−0.4	−1.6	+0.3	−0.80	−0.1	+2.3
Maximum changes after injection		−12.8	−12.0		−14.0	−2.5	−4.9	0.0	+0.7	−1.8	+1.65	−1.6	−3.1

Maximum changes during control period	+1.0	+1.1		+2.1	−0.7	+0.2	−0.4	−1.6	+0.3	−0.80	−0.1	+2.3
Maximum changes after injection	−12.8	−12.0		−14.0	−2.5	−4.9	0.0	+0.7	−1.8	+1.65	−1.6	−3.1

Dog 173

No. 3. Weight 20.0 kgm. Never before injected.
4 hour control period followed by intraperitoneal injection of fresh extract.
Dog had fasted for 20 hours and fasted throughout experiment.

After 20 hours, fast	58.0	32.5	27.2	30.2	36.5	14.6	15.0	12.8	14.4	9.35	6.60	11.4	11.4
After 21 hours, fast	58.0	33.0	27.0	30.3	37.4	14.7	15.0	12.7	14.3	9.35	7.80	12.2	10.5
After 22 hours, fast	54.0	32.3	27.0	30.0	36.7	14.5	15.0	12.6	14.1	9.15	6.90	12.8	11.1
After 23 hours, fast													
Just before injection	51.5	32.5	27.0		37.7	14.2				9.05	5.70	14.0	12.3
4 hours after injection	55.0	27.2	21.8	28.9	31.7					8.00	4.50	10.3	10.8
5 hours after injection	53.0	26.3	20.5	23.0	31.6		12.5			7.80	4.60	10.5	10.8
6 hours after injection	51.0	24.4	17.6	22.0	31.0	9.45	9.7			7.80	5.18	10.3	10.4
7 hours after injection	47.5	19.0	15.8	20.0	22.7	8.60	8.6	8.35	9.25	6.68	4.81	9.60	8.8
Maximum changes during control period		−0.8	−0.2	−0.2	−0.7	−0.4	0.0	−0.2	−0.3	−0.30	−2.10	+2.60	+1.80
Maximum changes after injection		−13.5	−11.2	−10.0	−15.0	−5.6	−6.4	−4.25	−5.5	−2.37	−1.20	−4.40	−3.50

tendency to rise a slight but definite drop in non-protein nitrogen and urea is observed during the fourth to sixth hours after injection. This evidence is by no means conclusive, but it does seem a fair indication that the disappearance of these substances from the blood cannot be entirely accounted to increased excretion.

Experiments to investigate the effect of anterior lobe extract upon the distribution in the blood of non-protein nitrogen, urea and amino acids. These experiments were performed in the hope of getting some further indication of the method of the disposal of the non-protein nitrogen constituents which disappear from the whole blood after injection. As has already been discovered by workers in other fields, the study of distribution between plasma and cells of blood constituents is not without difficulties. Direct analysis of the cells does not give dependable results, for it is impossible to get them entirely free from plasma by merely centrifuging the whole blood; and any washing of corpuscles would undoubtedly affect their composition. Also the measuring of cells presents mechanical difficulties.

For the normal distribution of the nitrogenous constituents of the blood, one may refer to Wu's (9) work upon this subject. We have used the methods recommended by Wu for precipitation and analysis of corpuscles and plasma. In the main, however, we have considered it more accurate to determine the corpuscle content by calculation from the whole blood, plasma, and haematocrit readings, rather than to depend upon the results of direct analysis. The trouble with this method is that any errors in plasma, or whole blood analysis or in haematocrit readings are magnified by the calculation. It is probably safest to use both methods before drawing any conclusions, and in a number of our experiments upon distribution the composition of the cells was determined both by direct analysis and by calculation.

The report of experiments in which the corpuscle content was determined by calculation only is given in table 11.

From these data and those given in tables 10 and 12 it may be concluded that as far as non-protein nitrogen and urea are concerned the disappearance from the blood after injection is *in general* fairly evenly distributed between the corpuscles and the plasma. But with the amino acids, the results obtained were rather surprising. A striking decrease in plasma amino acids appears to follow the injections, accompanied by an increase in the cell content. Examination of the data will show, however, that there are marked fluctuations in the plasma amino acids, and when the cell contents are determined by calculation only, the fluctuations would, of course, show up in these results also, but in the opposite direction. Since any changes in amino acids serve as a good indication that a true metabolic process is going on, we were particularly anxious to discover exactly how the amino acids are affected by anterior hypophyseal extracts. For this reason we

thought it worth while to devote some time to the direct analysis of whole blood, corpuscles and plasma, in order to make certain that these striking changes in plasma and cell amino acids were true findings.

The data given in table 12 show the results obtained. In these experiments heparin was used as an anticoagulent rather than lithium oxalate cloth, since it is said to have less osmotic effect upon the corpuscles and so make the haematocrit readings more dependable. A blank run upon the heparin showed that it did not contain appreciable amounts of nitrogen, urea or amino acids.

In these experiments the agreement between the figures on corpuscle content by analysis and by calculation is not very close. In the case of non-protein nitrogen and urea, however, the differences are sufficiently constant so that we may still feel justified in concluding that the drop in these constituents is quite evenly distributed between corpuscles and plasma.

But an examination of the data given in table 12 on amino acids indicates that something is wrong. Even in the control experiments when no injection is given, fluctuations are observed in the plasma amino acids and consequently in the corpuscles as determined by calculation. In the whole blood and in the cells by direct analysis, this does not occur. It is probable that the explanation for this rather puzzling observation lies in errors in the plasma analysis which are due to incomplete neutralization of the filtrates before addition of the color reagent. Although we have used only half volumes of sodium tungstate and sulphuric acid in precipitating plasma, as recommended by Wu (9) it is possible that even this is an excess and that the filtrate is consequently of rather high acidity. In our last analyses we had some indication that this was the case. After neutralizing all the filtrates with sodium carbonate, as is always done in amino acid determinations, we waited longer than usual before adding the color reagent, and noted that in the case of the plasma filtrates the pink color of the phenolphthalein had entirely faded. Tungstic acid, being a weak acid, is slow in giving an end point with sodium carbonate. We would therefore advise others in making amino acid determinations to allow plenty of time before adding the color reagent in order to make sure that neutralization is complete. It would probably also be better to use less sodium tungstate and sulphuric acid in precipitating plasma protein.

Our first observations, therefore, of fluctuations in plasma and cell distribution of amino acids following injection must be considered doubtful. In the first place the same fluctuations occur during control periods when no injections are given. And in the second place the direct analysis of the cells shows no such phenomenon but rather indicates that as with non-protein nitrogen and urea, the drop in whole blood amino acids represents a fairly equal loss from cells and plasma.

In the disposal of the non-protein nitrogen constituents which disappear from the blood after the injection of anterior hypophyseal extract, it may therefore be concluded that both plasma and cells are called upon.

It may be noted in these distribution experiments where haematocrit readings were always made that repeated withdrawal of blood specimens for analysis results in considerable decrease in corpuscle content. Since the blood volume of animals is kept constant, some blood dilution must occur and it might be questioned whether the fall in non-protein nitrogen constituents is not merely the effect of dilution. A number of answers

TABLE 13

Summary of results on whole blood

Amino acids

INJECTION EXPERIMENTS		CONTROL EXPERIMENTS		
Dog	Maximum drops after injection	Dog	Remarks	Maximum changes during control periods
160	−1.90	160	Inactivated extract	−0.65
161	−0.95	161	Inactivated extract	−0.50
161	−1.44	161	Pituitrin	−0.34
158	−0.80	174	No injection	−0.35
158	−1.45	172	No injection	−0.30
159	−1.72	170	Serum protein extract	+0.10
159	−1.43	173	No injection	−0.30
159	−2.00			
168	−1.13			
168	−0.65			
169	0.0			
170	−0.60			
171	−1.63			
French bull male	−1.30			
172	−1.80			
173	−2.37			

to this immediately suggest themselves, but the simplest lies in the examination of the data on control periods. The decrease in corpuscle content during the control period of experiment number three of table 12, for example, is extremely marked. However, there is no disappearance of the non-protein nitrogen constituents of the blood until after the injection. It is undoubtedly safe to conclude that the effect observed is not due to dilution.

In going over all the data on injection experiments, it may be observed that the drop in amino acids is sometimes slight. However, a comparison of the changes in whole blood amino acids caused by injections of active

TABLE 14

Blood analysis before injections were started, and after acromegaly was apparent

Dog 160. Control period

DATE AND WEIGHT	PHOSPHORUS		SUGAR	CALCIUM	CREATININE		URIC ACID	NON-PROTEIN NITROGEN	UREA	AMINO ACIDS
	Inorganic	Total			Preformed	Total				
10/22/28	4.45	25.8	87.5	10.80	1.29	3.32		28.0		
10/25/28	5.06	27.2	87.0	10.80	1.23	3.55		32.4		
15.4 kgm.										
10/30/28	4.55	29.6	93.0	13.35	1.20	3.36	1.05	34.3		
11/ 1/28	4.70	30.0	94.0	13.20	1.21	2.86	1.05	33.0		
11/ 5/28	4.55	29.6	88.5	13.60	1.20	2.86	1.00	33.7		
11/ 8/28	4.55	29.6	87.2	12.40	1.33	3.33	0.832	38.0		
15.8 kgm.										
11/13/28	4.45	29.4	95.5	11.60	1.46	3.08	1.05	37.0		
11/26/28	4.56	33.3	98.5		1.39	3.33	1.17	40.5		
16.2 kgm.										
Same dog nine weeks later after daily injection of active extract. Acromegaly apparent										
2/ 3/29	5.42	31.2	102	12.60	1.68	3.20	1.60?	46.2	20.0	11.2
2/ 7/29	5.33	30.7	95.0	12.60	1.66	3.25	1.33	39.2	17.2	9.62
22.4 kgm.										

TABLE 15

Blood analysis before injections were started, and after acromegaly was apparent

Dog 161. Control period

DATE AND WEIGHT	PHOSPHORUS		SUGAR	CALCIUM	CREATININE		URIC ACID	NON-PROTEIN NITROGEN	UREA	AMINO ACIDS
	Inorganic	Total			Preformed	Total				
10/22/28	5.64	25.6	81.0	12.35	1.36	3.45		27.7		
10/25/28	5.92	28.6	82.5		1.35	3.46		42.1		
18.1 kgm.										
10/30/28	6.00	33.7	78.5	12.70	1.29	2.83	1.16	37.4		
11/ 1/28	5.94	35.4	80.0	11.80	1.22	3.00	1.16	36.8		
11/ 5/28	6.15	35.6	79.0	13.20	1.15	3.00	1.13	37.8		
11/ 8/28	6.15	32.7	83.0	13.00	1.27	3.42	1.14	37.0		
17.3 kgm.										
11/13/28	5.80	31.2	79.0	12.60	1.43	3.42	1.00	38.8		
11/26/28	6.40	33.3	91.5	12.20	1.43	3.75	1.23	40.0		
17.9 kgm.										
Same dog nine weeks later after daily injection of active extract. Acromegaly apparent										
2/ 3/29	6.46	31.2	87.5	13.00	1.65	3.33	1.44	40.5	17.7	10.4
2/ 7/29	6.35	30.7	87.0	13.40	1.68	3.35	1.26	40.0	17.2	9.8
28.6 kgm.										

anterior lobe extract and those found in the control experiments (see table 13) seems to suggest that the growth principle of the pituitary has some effect upon the amino acids of the blood.

As has been pointed out above, this is particularly important as an indication of the endogenous nature of the observed effect of injections of anterior hypophyseal extracts upon the non-protein nitrogen constituents of the blood of dogs.

Daily blood analyses over a control period before injections were started and after acromegaly was apparent. On the two dogs that showed the best response to injections (that is, increase in weight, protrusion of lower jaw, acral enlargement, etc.) the analyses reported in tables 14 and 15 were made. The only differences observed in the composition of the blood during the control period and after acromegaly was established are possible slight increases in creatinine and uric acid. We do not feel, however, that these changes are sufficient to justify any conclusions, especially when only two such experiments were carried out.

SUMMARY

The blood of fasting dogs was analyzed just before and for a number of hours after the injection of growth promoting extracts of the anterior hypophysis of the ox.

During the experimental period no significant changes were found in total phosphorus, preformed or total creatinine, uric acid or sugar.

A drop in serum calcium following the injection was often but not always observed. Very slight changes in inorganic phosphorus sometimes occurred but the findings were not consistent.

A marked drop in non-protein nitrogen, however, was always observed after the injection of an active extract (20 to 30 per cent).

The disappearance of some urea and amino acids from the blood following injection was also an almost constant finding, the amount of drop in these constituents being fairly well in keeping with the percentage composition of the blood, and not sufficient to account for more than 70 per cent of the total drop in non-protein nitrogen.

Control experiments showed that after twenty hours of fasting a level of non-protein nitrogen, urea and amino acids is reached in the blood.

Pituitrin does not cause a disappearance from the blood of non-protein nitrogen, urea or amino acids. Neither does an extract of serum protein, nor an extract of anterior hypophysis which has been inactivated by boiling.

These control experiments indicated that the disappearance of non-protein nitrogen, urea, and amino acids from the blood following the injections of active extracts is probably due to the effect of the growth principle of these extracts.

The question arises as to whether or not this disappearance of non-protein nitrogen constituents of the blood may be attributed to increased excretion. It is impossible to draw any final conclusions upon this point, for the amounts of non-protein nitrogen and urea which disappear from the blood are too small to be traced in the urine. Experiments, however, in which the dog is deprived of water, thus cutting down on urine formation, still show appreciable drops in the non-protein nitrogen constituents of the blood after injection, and seem to furnish some evidence against increased excretion as the only explanation of the phenomena observed.

Also the fact that the amino acids and the "undetermined nitrogen" portion of the blood decrease after injection seems to indicate that the effect is at least in part of an endogenous nature.

The distribution between corpuscles and plasma of the non-protein nitrogen constituents of the blood is apparently not affected by the injection of active extracts of the anterior hypophysis of the ox. The disappearance from the blood of these constituents after injection is in general rather evenly distributed between corpuscles and plasma.

Total analysis of the blood of two dogs who had been made definitely acromegalic by daily injections of active extracts of anterior lobe, did not reveal any definite changes in the composition of their blood from what it had been during the control period before any injections had been given.

The results of the experiments carried out in the course of this investigation seem to supply some evidence that the growth promoting principle of the anterior hypophysis has an immediate effect upon the non-protein nitrogen constituents of the blood. It seems reasonable that this effect might be, at least in part, one of mobilization from the blood for the purposes of building up new protoplasm.

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STUDIES ON THE FUNCTION OF THE LUMBAR SYMPATHETIC OUTFLOW

I. The Relation of the Lumbar Sympathetic Outflow to the Sphincter Ani Internus

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Within recent years the rapid development of surgery of the sympathetic nervous system has emphasized the need of precise knowledge of its anatomy and physiology. We were led to undertake this series of studies by the suggestion that idiopathic dilatation of the colon (Hirschsprung's disease) be treated by removal of the lumbar sympathetic trunks with their ganglions and communicating branches. The physiologic principles on which this operation is based are sound, if Gaskell's conception of the innervation of the large bowel and its internal sphincter can be proved to be correct. According to Gaskell, through the lumbar outflow of the sympathetic system pass impulses which are inhibitory to the musculature of the large bowel and motor to the internal sphincter of the anus. The operation reported by Judd and Adson would thus leave motor nerves in less disputed control of the colon: according to Gaskell, these reach the large bowel by way of the sacral sympathetic outflow. At the same time, it would diminish the opposition to the expulsion of the contents of the bowel offered by the internal sphincter of the anus.

In the present paper we shall record a series of experiments performed on dogs which have convinced us that so far as the internal sphincter of the anus is concerned, the lumbar sympathetic outflow has the influence attributed to it by Gaskell. At this time we do not propose to review previous work on this subject; when we have reported our observations on other functions of the outflow, we shall be in a position to present a more fruitful discussion of the whole matter.

Anatomy. The connections of the inferior mesenteric ganglion of the dog with the lumbar sympathetic trunks have been studied by von Frankl-Hochwart and Frölich. Our dissections of material hardened in formalin bear out the description in their paper. Connector fibers pass to the gan-

glion from the second, third, and fourth lumbar ganglions of both sides, and a nerve bundle, often of considerable size, reaches it from the network of nerve fibers on the ventral aspect of the aorta. We have proved that the majority of those fibers in the roots of the ganglion which are concerned with the innervation of the internal sphincter of the anus have cell stations in the ganglion. From it, impulses controlling the sphincter pass, usually in postganglionic fibers, by three routes: along the lumbar colonic nerves, which form a thick strand accompanying the inferior mesenteric artery, and along the right and left hypogastric nerves.

A long median abdominal incision allows good exposure of the whole of the nervous apparatus from the lumbar trunks to the postganglionic fibers. We have found it easiest technically to approach both lumbar trunks from the left side, after incising the peritoneum along the outer side of the aorta and displacing the vessel to the right side.

METHODS OF EXPERIMENTS. In all our experiments we used adult dogs under ether anesthesia. The colon was washed out by an enema immediately before the experiment. To help to prevent contractions of the external sphincter of the anus from complicating our tracings, we gave the anesthetized animals divided doses of curare intravenously, in an amount sufficient to abolish respiratory movements. Thereafter anesthesia was continued by artificial respiration through an intratracheal tube. It has been maintained that the external sphincter is very resistant to the action of curare, but in the course of these experiments we observed uniformly that it was influenced by curare far more than we had expected. Tracings of the blood pressure were taken from the left carotid artery, whereas the right external jugular vein was exposed for the introduction of drugs.

For recording the tonus of the internal sphincter, we used a bulb of fine rubber secured to a glass tube of 5 mm. bore in such a way that when the bulb was introduced the glass tube lay in the grasp of the external sphincter, while the rubber bulb lay in the grasp of the internal sphincter. A strong rubber tube, with a side piece controlled by a clamp, passed from the glass tube to the proximal limb of a manometer. After experimenting with several fluids, we found that the manometer followed the changes of the sphincter in tension most accurately when it was filled with kerosene. After the bulb had been placed in position, it was inflated through the side piece to a pressure of 12 to 14 cm. of kerosene. The distal limb of the manometer was then connected to an air tambour, which recorded changes in tension on a slowly moving drum.

Our results could be obtained only when the tambour was placed as has been described; if it was introduced farther, so as to lie in the rectum, responses could not be demonstrated. Therefore, we feel that these must be attributed to the internal sphincter, and that our method excludes any complicating contractions of the musculature of the rectum.

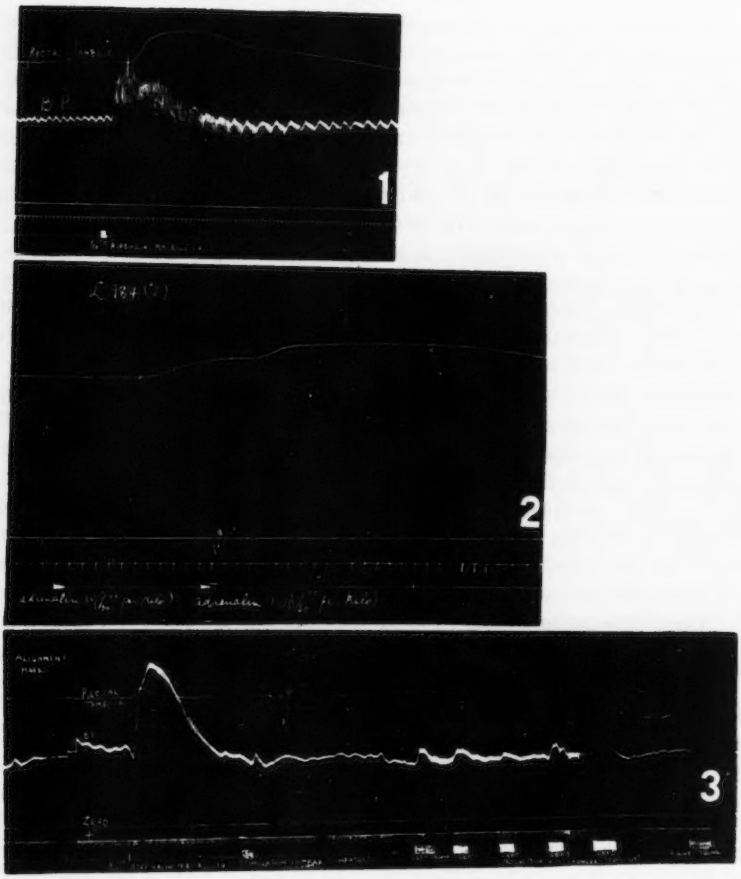

Fig. 1. Contraction of the sphincter after epinephrin intravenously. Toward the end of the contraction a tendency to rhythmic variations in tonicity is apparent. Time marker, four seconds.

Fig. 2. Summation of two contractions of the sphincter, both induced by epinephrin intravenously. The second dose was administered when the contraction resulting from the first was at its height. Time marker, four seconds.

Fig. 3. Contraction of sphincter on stimulation of either lumbar sympathetic trunk.

RESPONSE OF SPHINCTER TO EPINEPHRIN. The intravenous injection of epinephrin is well known as a convenient test for sympathetic innervation. The dose we used was 0.02 cc. of a 1:1000 solution of epinephrin

for each kilogram of body weight, diluted with 5.0 cc. of water. Such a dose caused well marked contraction of the sphincter (fig. 1) after a latent

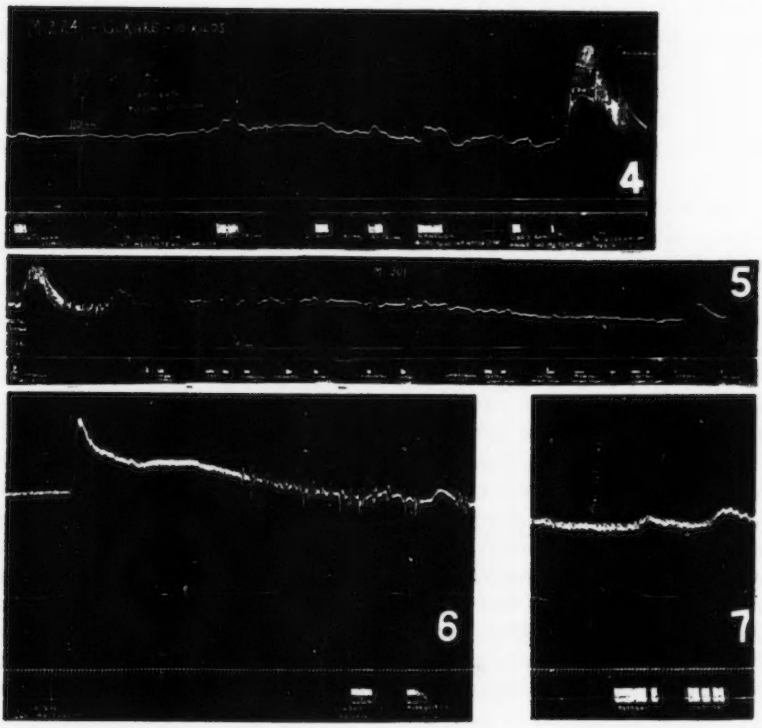

Fig. 4. Absence of contractions on stimulation of either lumbar sympathetic trunk, after nicotine solution 0.5 per cent had been painted on the inferior mesenteric ganglion. Time marker, four seconds.

Fig. 5. Contraction of sphincter on stimulation of hypogastric and lumbar colonic nerves. Contraction on stimulating peripheral segment of cut lumbar colonic nerve; no contraction on stimulating central segment. Time marker, four seconds.

Fig. 6. Record after ergotoxin intravenously. No contraction of sphincter on stimulating hypogastric or lumbar colonic nerves. Only a very slight contraction after epinephrin intravenously. Time marker, four seconds.

Fig. 7. Record after ergotoxin intravenously. Relaxation of sphincter on stimulation of hypogastric nerve, after sphincter was allowed to recover more of its normal tone. Time marker, four seconds.

period varying from fifteen to thirty-five seconds. The contraction attained its full height in from thirty to sixty seconds, and the high level was maintained for a considerable time, in one case for as long as two

hundred fifteen seconds. Often we found that after the injection of epinephrin, the tonus of the contracted sphincter varied in a rhythmic manner; these waves of contraction and relaxation had an almost constant periodicity of from twelve to fourteen seconds, irrespective of the size of the animal. It was possible to superimpose a second contraction on the first, by giving a second dose of epinephrin while the first contraction was at its height. The second contraction began after a shorter latent period than the first, and the total duration of the two contractions was increased (fig. 2.)

The response of the sphincter to an intravenous dose of epinephrin was so invariable that in all our work we produced it at the beginning and end of each experiment, as a test of the working of our recording apparatus.

RESPONSE OF SPHINCTER TO STIMULATION OF LUMBAR SYMPATHETIC TRUNKS. Stimulation of either lumbar sympathetic trunk with a faradic current was found to give rise to contraction of the sphincter (figs. 3 and 4). As was to be expected, we found that the more cranial the point stimulated, the more powerful was the resulting contraction, owing to the greater number of roots of the inferior mesenteric ganglion affected by the current. The results of stimulation of the lumbar trunks after the application of 0.5 per cent solution of nicotine to the inferior mesenteric ganglion varied; in most experiments there was no contraction of the sphincter, and in others only a slight one (figs. 3 and 4). This observation would appear to confirm Gaskell's contention that "the internal sphincter ani muscle is composed of two parts, of which one is supplied by motor and inhibitory neurones, which have traveled out together in the thoracico-lumbar outflow and are situated near the muscle, and the other is supplied by motor neurones belonging to the thoracico-lumbar outflow and situated in the inferior mesenteric ganglion." The majority of the fibers supplying the sphincter would appear from our results to belong to the latter group.

RESPONSE OF SPHINCTER TO STIMULATION OF LUMBAR COLONIC NERVES. We found that faradic stimulation of the lumbar colonic nerve, as it coursed in the mesocolon, gave rise to a well marked contraction of the sphincter. If the nerve bundle was completely divided, then stimulation of its peripheral segment led to a characteristic contraction, whereas stimulation of its central segment did not cause a contraction (fig. 5).

RESPONSE OF SPHINCTER TO STIMULATION OF HYPOGASTRIC NERVES. Faradic stimulation of either hypogastric nerve gave rise to a well marked contraction of the sphincter (fig. 5). This was always greater than the contraction obtained by stimulating the lumbar colonic nerve under the same experimental conditions.

RESPONSE OF SPHINCTER AFTER ADMINISTRATION OF ERGOTOXIN. In our search in the lumbar outflow for inhibitory nerves to the sphincter, we took advantage of an observation by Dale, that ergotoxin paralyzes the

motor nerves of the sympathetic system but does not affect the inhibitory nerves. When investigating this point we did not give curare, as in our other experiments. By inducing very deep ether anesthesia, we placed the internal sphincter at rest, as was indicated by a level base line on the recording drum. The sphincter was found to react normally to epinephrine intravenously and to stimulation of the lumbar colonic and hypogastric nerves.

We then gave an intravenous dose of 0.0005 mgm. of ergotoxin in watery solution for each kilogram of body weight. We found that after such a dose of ergotoxin, the sphincter did not contract after the intravenous administration of epinephrin or after faradic stimulation of the lumbar colonic or hypogastric nerves (fig. 6). The motor component of its nerve supply was therefore paralyzed. We were unable to cause relaxation of the sphincter on stimulation of these nerves, until we allowed it to recover some of its tone by lessening the depth of the anesthesia. It was then found that stimulation of the hypogastric nerves caused definite relaxation of the sphincter, whereas stimulation of the lumbar colonic nerves did not produce this relaxation (fig. 7). It would appear that those inhibitory nerves which reach the sphincter from the lumbar sympathetic outflow pass by way of the hypogastric nerves.

SUMMARY AND CONCLUSIONS

1. A series of experiments on the relation of the lumbar sympathetic outflow to the sphincter ani internus is described.

2. The sphincter derives a certain amount of motor innervation through the lumbar sympathetic outflow.

3. The majority of the fibers concerned are postganglionic fibers arising in the inferior mesenteric ganglion.

4. A few motor fibers are not interrupted in the inferior mesenteric ganglion.

5. Inhibitory fibers from the lumbar sympathetic outflow pass to the sphincter by way of the hypogastric nerves.

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EXPLANATION OF WEDENSKY INHIBITION

Part II. Explanation of "Paradoxes Stadium" in the Sense of Wedensky

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From the results of the various kinds of experiments described in part I (16b) we came to the conclusion that in complete inhibition the first nerve impulse alone reaches the muscle and that the subsequent impulses vanish at the beginning of the narcotized area, about 3 to 5 mm. inside of the upper wall of the narcotizing chamber.

As was stated in the previous part, at a certain stage of narcosis tetanic stimuli of great frequency, being applied at the upper, normal part of nerve, produce "complete inhibition"[1], whereas stimuli of less frequency evoke a continued tetanus. This is the essence of the so-called Wedensky effect. Let us call this phenomenon the "frequency factor" in order to distinguish it from another factor, i.e., "intensity factor". At first we will proceed to deal with the frequency factor.

For this purpose let us take tetanic stimuli of a given frequency and consider first the reason how and why the series of subnormal impulses vanishes in complete inhibition. The study of this question will lead of itself to the explanation of the frequency factor.

"*Frequency factor*". It is well known that for the production of "complete inhibition" by applying tetanic stimuli of given frequency a certain depth of narcosis is necessary. What is then the relation between them? This question is of fundamental importance, because by studying it closely we can see into the true nature of complete inhibition and so we may get the key to solve the "paradoxes Stadium" in the sense of Wedensky. To answer this question it is necessary, at first, to study the recovery process of normal as well as narcotized nerve.

1. On the recovery of nerve. Some years ago it was reported by us (17) that, contrary to Lucas (18) the refractory period of nerve is prolonged by

[1] We say "complete inhibition" only when the first impulse alone reaches the muscle, all the succeeding impulses being extinguished in the narcotized region.

narcosis, in other words the recovery is slowed by narcosis. This point will easily be understood by mapping a recovery curve of nerve, that is, a curve relating the strength of second stimulus to the least interval at which the muscle will give summated contraction. The curve *CDE* in figure 7 represents the recovery curve of a normal nerve-muscle preparation mapped by applying two successive stimuli at a point of nerve *B* (before narcosis) by means of the Lucas pendulum (see fig. 8). The first stimulus is fixed at a strength which is somewhat above maximal. The strength of the second stimulus is varied by changing the resistance, *W*, inserted in the primary circuit, the coil distance being fixed to give the threshold strength when there are 100 ohms in the primary circuit. It will be seen in figure 7 that the least interval becomes gradually shorter as the second stimulus is strengthened, till at last it reaches a constant value (α) when the resistance in the primary circuit is about 10 ohms. For simplicity we will call this constant value (α), to be obtained by such strength of the second stimulus,

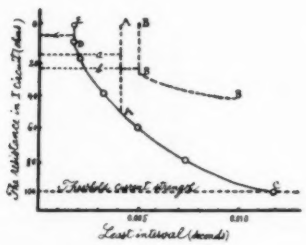

Fig. 7

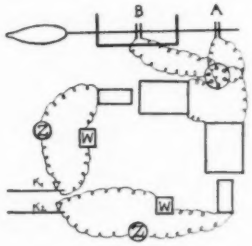

Fig. 8

"least interval" hereafter. We have proved elsewhere (19) that *DE* and *CD* represent respectively the absolute and the relative refractory period of the nerve, in this case, of the point *B*, at least when the nerve muscle preparation is above 15°C. So far it is the recovery curve of a normal nerve.

On narcotization this curve suffers a change. The recovery curve of *B* (narcotized part) moves gradually to the right and upwards as represented by *BBB* in figure 7. This indicates prolongation of the absolute refractory period (from α to b) and the heightened threshold in the relative refractory period of nerve. Figure 9 shows this point very clearly. As narcosis proceeds the curve approaches the horizontal line.

As to the recovery curve determined at the normal part A, outside the narcotizing chamber (fig. 8), the relation is quite different. Before narcosis it gives, of course, the same recovery curve *CDE* (fig. 7) as that of *B*, indicating that both points *A* and *B* take the same course of recovery after excitation. But by narcotizing the part of nerve between *A* and the muscle

the vertical part *DE* alone moves, as narcosis proceeds, gradually to the right as represented by *CAA* (fig. 7). Since the stimulated point *A* (normal part) is not affected at all by narcotization the second stimulus would set up an impulse at *A* just in the same relation as before narcosis. Consequently the lower part of the curve remains unchanged: the second impulse reaches the narcotized region late enough to pass through it. Only the earliest of second impulses are hindered from passing through because they reach the narcotized region too early when it is not yet sufficiently recovered from the first impulse. Therefore the least interval must be made greater (from α to a), if the second impulse is to pass successfully through the narcotized region where the recovery process is slowed as represented by the curve *BBB*.

It must not be forgotten that the prolongation of the least interval at *B* (narcotized part) has an utterly different meaning: it indicates the prolongation of the refractory period of the narcotized region as stated above.

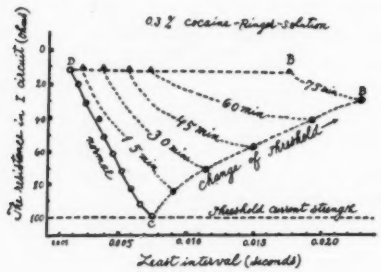

Fig. 9

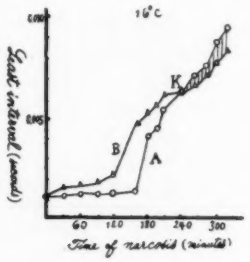

Fig. 10

The interval between stimuli must be made greater, if the second stimulus is to set up an impulse, because the recovery from the first impulse is slowed by narcotization. If the second impulse is once set up, it cannot fail to reach the muscle, passing the narcotized area without decrement.

2. The depth of narcosis necessary to produce complete inhibition. As the narcosis deepens, the least intervals at *A* and at *B* become gradually longer, although their prolongation has different meanings. What is the relation between the prolongation of these two least intervals? And what relations are there between their prolongation and the production of "complete inhibition"? To meet this question the change of the least intervals at *A* and at *B* was followed, on one hand, through the whole course of narcosis employing the method above described (see fig. 8), and on the other hand the tetanic stimuli of 200 frequency per second were applied at the outside electrode to detect the depth (time) of the narcosis at which "complete inhibition" appeared. One example from twelve experiments which were

made with the sciatic nerve is shown below (table 8 and fig. 10). Before narcosis A and B showed least intervals of the same value (1.1σ). The narcosis (0.2 per cent cocaine-Ringer solution) was started and in the early stage the least interval at B increased more rapidly than at A, for instance at 120 minutes of the narcosis the former is increased to 2.1σ, whereas[2] the latter is only 1.3σ. But from certain stage of narcosis this relation changed and the latter showed a more rapid increase and overtook the former. It will be seen in figure 10 that the curves A and B cross (K) at 240 minutes giving the same interval of 6.5σ each. After the crossing the increase in the least interval is much more marked at A than at B. The other experiments gave similar results.

The same experiments were repeated with N. abdominalis (unbranched nerve). As an example the result of experiment 2 which was made with 1.0 per cent urethane-Ringer solution is given in table 9 and figure 11. Ten experiments showed the same result without exception.

TABLE 8

16°C

Time of narcosis (minutes)	Least interval at A (σ)	Least interval at B (σ)
(normal) 0	1.1	1.1
30	1.1	1.4
60	1.2	1.8
90	1.2	1.9
120	1.3	2.1
150	1.5	4.8
180	4.3	5.3
200	4.7	5.6
220	5.6	6.1
240	6.5	6.5
260	7.1	6.9
280	7.7	7.5
300	8.4	8.0
320	9.6	8.7

TABLE 9

12°C

Time of narcosis (minutes)	Least interval at A (σ)	Least interval at B (σ)
(normal) 0	1.8	1.8
30	2.0	2.2
60	2.6	2.8
90	3.1	3.4
120	3.6	3.8
150	4.4	4.2
180	4.7	4.5
210	5.5	5.0
240	6.1	5.7

What is most remarkable in these experiments is that the complete inhibition can be produced only after the stage of narcosis at which the least interval at A overtook that of B, in other words, only after the crossing of the curves A and B, and never before. This is true not only when tetanic stimuli of 200 frequency per second are used to produce complete inhibition, but also for stimuli of any other frequency. Numerous experiments were made on this point with stimuli of various frequencies. In order to vary the frequency, tuning forks of various vibration (20, 50, 100 and 500 per second) were used to insert in the primary circuit (the coil distance was so made that the break as well as the make shock was strong enough, acting alone, to provoke a maximal contraction of the muscle). In one of the experiments the curves crossed when the least interval increased to only 4.5σ (K in fig. 10). The crossing in such an early stage of

[2] The determination is usually made first at A and then at B. During this time the narcosis deepens. Therefore the actual difference may be somewhat smaller.

narcosis is very rare. In most of the experiments they crossed between 5.5 and 6.5σ. We saw only one case in which they crossed as late as at 7.0σ. In none of 28 experiments was complete inhibition produced before the crossing, however frequent stimuli might be employed. This result is of fundamental importance for the explanation of complete inhibition. But before we proceed further on this point it is necessary to consider a while the reason why the curves *A* and *B* cross in this way.

3. On the reason why the curves A and B cross. Before narcosis the two least intervals are the same at *A* and *B*, simply because both of them are the absolute refractory period (20) of the normal nerve: the earliest second impulse, if once set up, cannot fail to produce summated contraction of the muscle.

In the early stage of narcosis the least interval at *A* (normal part) is smaller than that at *B* (narcotized part), which indicates, as previously mentioned, the prolonged absolute refractory period of the narcotized nerve (see figs. 10 and 11). This point may need some explanation. The works of Forbes, Ray and Griffith (21) and of Gasser and Erlanger (22), confirmed in this laboratory (23) show that the rate of conduction of the second (subnormal) impulse is less than that of the first (normal) one and that the second impulse increases gradually in size during its propagation, finding the nerve more and more recovered as it travels. Therefore the second impulse started from *A* (upper normal part), though set up after the first within a shorter interval than the absolute refractory period of the narcotized part, suffers some delay during its propagation to the narcotized part and so arrives there after the absolute refractory period due to the first impulse is over. In the experiments shown above (figs. 10 and 11) the distance between the electrode *A* and the upper chamber wall was 15 to 25 mm., according to the length of the nerve used. To confirm this reasoning we will show a result of the experiments in which the upper (normal) part was examined at two different points *A* and *A'* (table 10). The electrodes *A* and *A'* were respectively 30 and 15 mm. distant from the upper wall of the narcotizing chamber. It will be seen that in the early stage of narcosis the least interval is smaller at *A* (far electrode) than at *A'* (near electrode). This is simply because the second impulse started from the far electrode suffers a greater delay than that started from the near electrode.

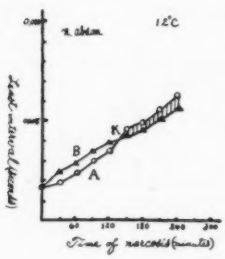

Fig. 11

From this view it is easily understood how the two curves *A* and *B* cross. Let us consider that stage of narcosis at which the crossing of the curves just occurs (see *K* in fig. 10). At this stage (240 minutes) the least

intervals are equal (6.5σ) at A and at B. At A the first and the second impulses are set up at just the same interval as the absolute refractory period of the narcotized part. Consequently the second impulse, suffering some delay during its conduction, reaches the narcotized part after the absolute refractory period due to the first impulse is over, in other words, the second impulse falls into the *relative* refractory period of the narcotized part, and yet it is doomed to vanish, as the later parts of the curves indicate. Therefore we can safely conclude that at least the second impulses evoked in the shaded area between the two curves, A and B, are extinguished in the beginning of the narcotized region by falling into the relative (not absolute) refractory period left by the first (see figs. 10 and 11). Strictly

TABLE 10

0.2 per cent cocaine-Ringer-solution. 15°C.

TIME OF NARCOSIS	LEAST INTERVAL		
	At A(σ) (30 mm. central)	At A'(σ) (15 mm. central)	At B(σ) (narcotised part)
minutes			
(Normal) 0	1.1	1.1	1.1
45	1.1	1.7	2.9
80	1.1	2.8	3.3
100	1.4	3.1	3.7
120	3.2	3.3	3.9
150	3.5	3.5	4.4
170	4.0	4.0	4.6
190	4.8	4.8	4.9
210	5.8	5.8	5.8
230	6.5	6.5	6.2
245	7.1	7.1	6.9
260	7.8	7.8	7.6
280	8.8	8.8	8.0
300	10.5	10.5	8.6
315	14.4	14.4	10.3

speaking, the same effect will occur in the stage just before crossing. But in this advanced stage of narcosis the second impulse set up at A is large in size (the stimulus interval is great), and so the delay which the second impulse may suffer till it reaches the narcotized part is very small compared with that in the early stage of narcosis. Therefore it is not far from truth when we say, for the sake of simplicity, that the crossing point K represents the critical stage of narcosis and that at this stage for the first time the second impulse is extinguished by falling into the relative refractory period. There is no wonder that this effect would occur in the advanced stage of narcosis, because, as it was shown in figure 9, the part of the recovery curve representing the relative refractory period (BB in fig. 9) becomes

almost parallel to the abscissa in the advanced stage: this means that even in the relative refractory period a very strong stimulus is needed to excite the narcotized nerve. Therefore those second impulses which are below the threshold of the narcotized part at this moment (in its relative refractory period) would be extinguished by falling into the relative refractory period.

4. Production of complete inhibition with stimuli of various intervals. From the observations above described it became clear that the curves *A* and *B* cross because the second impulse starting from *A* (outside electrode) vanishes in the narcotized part, falling into the relative refractory period due to the first impulse, and that complete inhibition is produced only after the crossing, no matter how frequent the stimuli might be. It fo lows therefore that for the production of complete inhibition the abolition of the second impulse in the relative refractory period is necessary. However, this point needs further confirmation, because in the previous experiments in which the tuning forks or the interrupter (Zimmermann) were used to obtain the tetanic stimuli there is some ambiguousness as to the interval between the nerve impulses entering into the narcotized region. We saw, by examining the action current, that the nerve impulses evoked by that method are not always regular in size and in interval, especially when a tuning fork of 500 vibrations per second is used. To avoid this source of error we have employed a Helmholz pendulum, by means of which six successive stimuli could be applied at exactly timed intervals and at any desired strength. The experimental arrangement is diagrammatically reproduced in figure 12. The strength of the each stimulus was always so adjusted that each one, being fully maximal, was effective in producing summated muscular contraction when applied successively; therefore, as to the height of muscle contraction

$$\text{first stimulus alone} < \text{first} + \text{second} < \text{first} + \text{second} + \text{third}$$

and so on.

a. Complete inhibition by successive stimuli of 5.0σ interval. As an example the result of experiment 3 (table 11) will be explained. Before narcosis the contraction of the muscle by the six successive stimuli and by the single maximal stimulus was respectively 60 and 40 mm. in height. In narcosis both of them decreased and at 130 minutes they became equal in height (28 mm.). This is no other than complete inhibition. In this stage the least interval at *A* and at *B* was increased respectively to 5.4 and to 4.9σ. Thus the complete inhibition was produced just after the crossing. Of course we had many cases in which the crossing did not occur so early as in this example—sometimes as late as at a 6.5σ interval. In such cases complete inhibition was never produced before the crossing, although the stimuli interval was kept 5.0σ as before. We had to wait till the least

interval at A overtook that of B. This point is of great interest and needs further confirmation.

b. This point is well confirmed by the following experiment in which two series of stimuli of different intervals, 3.0 and 6.0σ, were used. Table 12 shows an example. At 75 minutes of narcosis the least intervals were increased to 4.2σ at A and 5.3σ at B, and so the crossing has not yet occurred. The complete inhibition is not yet produced by either of the two series of the stimuli; that it is not produced by the stimuli of 6.0σ interval is a matter of self-evidence because at this stage even the second stimulus is effective to evoke a summated contraction. But, as to the stimuli of 3.0σ interval, the reason is somewhat different. The second impulse vanishes falling into the absolute refractory period left by the first, and the third impulse becomes again effective. Thus the muscle contraction is equal in height by the two series of stimuli (23 mm.). At 90 minutes complete

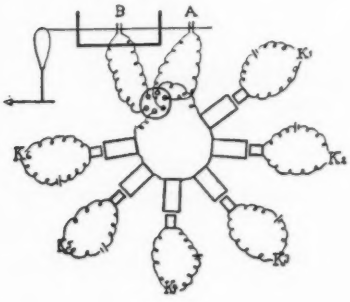

Fig. 12

TABLE 11

0.5% cocaine-Ringer solution 12°C

Time of narcosis (minutes)	Least interval at A (σ)	Least interval at B (σ)	Contraction height by 50 g load (mm)	Contraction height by 100 g load (mm)
(normal)	2.0	2.0	6.0	4.0
30	2.4	3.1	5.6	3.8
90	3.7	4.0	5.4	3.5
120	4.6	4.7	3.5	3.0
130	5.4	4.9	2.8 complete	2.8

inhibition was produced by both series of stimuli at the same time. At this stage the crossing was just over, the least intervals being 6.8σ at A and 5.7σ at B. The second impulse from the stimuli of 6.0σ interval, and the third impulse from the stimuli of 3.0σ interval fall equally in the shaded area (fig. 10), i.e., in the relative refractory period due to the preceding impulse, and vanish, leaving behind an "after-effect" to inhibit the succeeding impulse.

As to the "after-effect", we will deal with it later in detail.

c. Complete inhibition by successive stimuli of 7.0σ interval. The result of experiment 2 is given in table 13 as an example. At 150 minutes of narcotization the least interval is increased to 6.0σ at B and to 6.4σ at A. Therefore the crossing has already taken place. But the complete inhibition is not yet produced, the contraction by six stimuli being still higher than that evoked by the single stimulus. It is a matter of course because at this stage in which the least interval at A is only 6.4σ, the second impulse which

is evoked 7.0σ after the first is able to pass through the narcotized region. The complete inhibition was produced at 170 minutes when the least interval increased at *A* to 7.6σ, and at *B* to 6.5σ.

d. The experiments were made with successive stimuli of 10.0σ interval too. But we will be content here with saying that complete inhibition

TABLE 12

0.4 per cent cocaine-Ringer-solution. 15°C.

TIME OF NARCOSIS	LEAST INTERVAL		CONTRACTION HEIGHT		
	At A (σ)	At B (σ)	By 3.0 σ stimulus	By 6.0 σ stimulus	By single stimulus
minutes			*mm.*	*mm.*	*mm.*
(Normal) 0	1.4	1.4	55		25
20	1.8	2.1	35		21
40	2.7	2.9	29		19
75	4.2	5.3	23	23	17
85	—	—	17	17	15
90	6.8	5.7	13	13	13
			Complete inhibition	Complete inhibition	

TABLE 13

0.5 per cent cocaine-Ringer solution. 15°C.

TIME OF NARCOSIS	LEAST INTERVAL		CONTRACTION HEIGHT	
	At A (σ)	At B (σ)	By 7.0 σ stimulus	By single stimulus
minutes			*mm.*	*mm.*
(Normal) 0	1.8	1.8	56	38
30	2.0	2.4	54	35
60	2.6	3.1	39	24
120	4.7	5.2	30	19
150	6.4	6.0	21	17
170	7.6	6.5	15	15
			Complete inhibition	

TABLE 14

0.5 per cent cocaine-Ringer solution. 13°C.

TIME OF NARCOSIS	LEAST INTERVAL		CONTRACTION HEIGHT		
	At A (σ)	At B (σ)	By 5.0 σ stimulus	By 10.0 σ stimulus	By single stimulus
minutes			*mm.*	*mm.*	*mm.*
(Normal) 0	2.0	2.0	56		35
30	2.7	3.2	53		31
60	4.2	4.4	34		25
90	5.3	4.8	21	32	21
140	6.3	5.7	Complete	20	16
160	8.0	7.5	inhibi-	16	14
180	11.8	9.8	tion	14	14
				Complete inhibition	

could be produced only after the least interval at *A* increased over 10.0σ, although the crossing occurred, in most cases, between 5.0 and 6.5σ.

e. One example will be shown in table 14, in which two series of stimuli of 5.0 and of 10.0σ intervals were examined in the same preparation. With stimuli of 5.0σ interval the complete inhibition was produced after 90

minutes of narcotization when the least intervals at A and at B were respectively 5.3 and 4.8σ. At this stage stimuli of 10.0σ interval still produced a tetanus. It was at 180 minutes that complete inhibition was produced. The least interval was increased to 11.8σ at A and 9.8σ at B at that moment. Figure 13 illustrates the condition at 90 minutes of narcotization when complete inhibition was first produced by the stimuli of 5.0σ interval. Vertical lines AA and BB represent respectively the least intervals at A and at B, and the arrows indicate the series of stimuli. The second stimulus (impulse) falls in the shaded area between AA and BB. This is extinguished and leaves behind an "after-effect" which abolishes the succeeding impulses. Thus the complete inhibition is produced by stimuli of 5.0σ interval. On the other hand, if stimuli of 10.0σ interval are used at this stage of narcosis, then the second stimulus (impulse) falls to the right of AA and so it can of course reach the muscle to produce summated contraction.

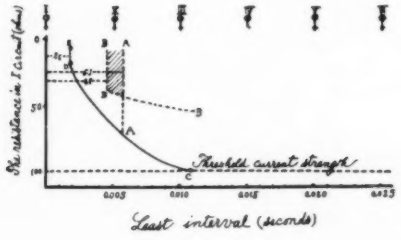

Fig. 13

The stimuli of 5.0 and 10.0σ intervals represent frequencies of 200 and 100 per second respectively. Therefore we may express the relation above described as follows: at a certain stage (90 minutes) of narcosis the stimuli of great frequency (5.0σ interval) produce complete inhibition, whereas the stimuli of less frequency (10.0σ interval) produce tetanus. This is no other than the "frequency factor" as defined in the beginning of this part. The detailed description will be given in section 7 (p. 704).

5. Conditions necessary to produce complete inhibition. It will be seen from the observations above described that for the production of complete inhibition the following factors are needed:

1. The stage of narcosis in which the least interval of A is increased over that of B, i.e., the crossing of the curves A and B (see fig. 10). Until this stage of narcosis is reached, the impulse is not extinguished in the relative refractory period due to the first impulse.

2. That the least interval of A is so increased that it is greater than the stimulus interval. Otherwise the second impulse can reach the muscle to make summated contraction.

3. That the second stimulus (impulse) falls earlier than the least interval of A but later than that of B, i.e., that it falls in the shaded area between AA and BB in figure 13. If it falls to the left of BB, it vanishes in the absolute refractory period due to the first impulse and leaves behind no after-effect. Consequently, if the third stimulus happens to fall to the right of AA, then this can reach the muscle to produce summated contraction. On the contrary, if the third stimulus happens to fall in the shaded area, then this vanishes leaving behind an "after-effect" to inhibit the succeeding impulses. Therefore, when the second impulse falls to the left of BB, the result varies according to the relation between the stimulus interval and the width of the shaded area. Thus it can happen that stimuli of less frequency (200 per sec.) produce complete inhibition, whereas in the same stage stimuli of greater frequency (300 per sec.) do not. This apparently strange effect was sometimes actually met with.

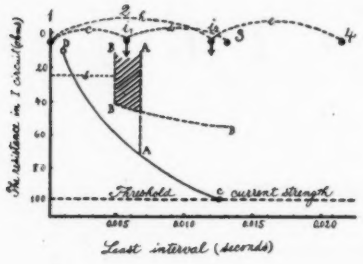

Fig. 14

TABLE 15

0.3% Cocaine-Ringer Solution

Time of period (seconds)	Least interval at A (σ)	Least interval at B (σ)	c (σ)	d (σ)
(normal)	1.1	1.1		
40	1.6	4.3		
60	4.2	5.0		
75	5.2	5.6		
100	6.2	6.0	6.0	7.5

6. *The measurement of the "after-effect."* The next problem is to measure the duration of the "after-effect" which the second impulse vanishing in the shaded area (relative refractory period due to the first impulse) leaves behind. To meet this question three keys in a Lucas pendulum were employed and the second stimulus (*2* in fig. 14) was used as an interpolated stimulus, i_1. It was so timed that the interpolated stimulus fell in the shaded area, and the shortest interval, d, was determined at which the third stimulus, *3*, became just effective in producing a muscular summation, applying three stimuli (the first, i_1 and *3*) successively at A, the outside electrode. Each stimulus was made ten times as strong as the threshold strength for the normal nerve. One example (expt. 4) will serve to explain the result obtained (table 15). In this case the determination was made as soon as the curves A and B crossed. The interval between the first and the interpolated stimulus i_1 (this interval is shown by c in fig. 14 and in table 15) was made 6.0σ, so that i_1 fell in the narrow shaded area just after the crossing. Of course i_1 itself vanished without reaching the muscle, but left an after-effect, d, lasting as long as 7.5σ; in other words, the third stimulus

became just effective to produce a summated contraction, being applied at 7.5σ after the interpolated stimulus i_1, and not before. It will be noted that d (after-effect) was greater than b (absolute refractory period of the narcotized part at the moment, see fig. 14). The ratio d/b was $7.5/6.0 = 1.25$ in this case.

As narcosis deepens, b gradually increases, as indicated by the increase of the least interval at B. However, d increases more rapidly than b and so the ratio d/b becomes gradually greater as narcosis proceeds. This point is clearly shown in the result of experiment 10 (table 16). In this experiment the after-effect, d, was determined four times at four different stages of the narcosis, at 110, 130, 160 and 180 minutes. During this time, b (absolute refractory period of the narcotized part) increased from 4.9 to 5.8σ, and d (after-effect) from 6.0 to 16.3σ. The ratio increased from

TABLE 16

0.3 per cent cocaine-Ringer-solution. 14°C.

TIME OF NARCOSIS	LEAST INTERVAL		(i) INTERPOLATED STIMULUS			
	At A (σ)	At B (σ)				
minutes						
(Normal) 0	1.5	1.5				
70	1.7	2.7	c (σ)	d (σ)	Ratio d:b	Ratio d:c
80	2.0	4.6				
90	4.6	4.8				
110	6.0	4.9	5.5	6.0	1.2	1.1
130	6.8	5.4	6.3	6.8	1.3	1.1
160	8.0	5.5	7.0	10.3	1.9	1.5
180	8.5	5.8	7.4	16.3	2.8	2.2

1.2 to 2.8. It is important to know that in each stage d lasted longer not only than b, but also than c (the interval between the first and the interpolated stimulus, see fig. 14).

The other 23 experiments gave similar results. Now, suppose that in figure 14 a series of stimuli recurring regularly at c interval each is applied at A, the outside electrode. It is evident that if the second stimulus (impulse) falls in the shaded area, then it leaves behind an after-effect which is long enough to inhibit the third, because the after-effect, d, lasts longer than the stimulus interval, c. Therefore, if this third (extinguished) impulse gives rise, in its turn, to a new after-effect longer than c, then the fourth impulse would also be extinguished and so on. This point was tested in following way (see fig. 14): after the least interval of A overtook that of B, i_1, was interpolated to fall in the shaded area and d was deter-

mined as before. Then i_2 was interpolated somewhat earlier than the end of d (i.e., within d), and the shortest interval, e, was determined at which the fourth stimulus, *4*, became just effective to produce muscular summation by applying four stimuli *1*, i_1, i_2 and *4* successively at A. Of course, i_1, and i_2 do not reach the muscle. One example (expt. 14) will serve to explain this relation (table 17). It will be seen that i_2 leaves an after-effect, e, lasting much longer than c, and also longer than d due to i_1 at the moment, and that both after-effects, d and e, increase as the narcosis deepens.

Thus we have seen the reason why the fourth stimulus in the above mentioned series of stimuli cannot affect the muscle. It will not be necessary to describe the experiment showing that the fourth inhibits the fifth in the same manner.

TABLE 17

0.3 per cent cocaine-Ringer-solution. 15°C.

TIME OF NARCOSIS	LEAST INTERVAL		i_1 I INTERPOLATED STIMULUS			i_2 II INTERPOLATED STIMULUS		
	At A (σ)	At B (σ)	c (σ)	d (σ)	Ratio d:c	h interval ($1-i_2$) (σ)	e (σ)	Ratio e:c
minutes								
(Normal) 0	1.6	1.6						
30	1.7	2.7						
80	2.3	3.6						
90	4.5	4.8						
110	6.2	4.8	5.7	6.4	1.1	11.5	9.9	1.5
130	6.8	5.3	6.0	7.3	1.2	12.5	12.6	2.1
160	8.1	5.7	7.2	11.5	1.6	17.0	16.9	2.3
180	8.2	6.5	7.5	15.3	2.0	22.0	20.8	2.8

7. The explanation of "frequency factor." For the sake of convenience let us take the example shown in table 14 (p. 700) in which two series of stimuli of different interval (5.0 and 10.0σ) were used in the same preparation. Figure 15 represents the conditions at 90 minutes of narcotization when complete inhibition was first produced by the frequent stimuli (5.0σ), whereas the less frequent stimuli (10.0σ) still evoked a tetanus. At first we will deal with the stimuli of 5.0σ interval. The white and black circles indicate respectively effective and ineffective stimuli (impulses). The first (normal) impulse reaches the muscle. The second falls in the shaded area between AA and BB and vanishes on account of the heightened threshold of the relative refractory period due to the first normal impulse (see fig. 15). This second impulse, although itself vanishing, leaves behind an after-effect, d, lasting longer than c (5.0σ). Conse-

quently the third coming at 5.0σ after the second falls within d and vanishes too. This third, itself vanishing, leaves behind a new after-effect, e, which lasts longer not only than c but also than d. The fourth falls, therefore, within e and vanishes and so on. Thus a series of stimuli at a frequency of 200 per second (the interval is 5.0σ) produces complete inhibition, the first (normal) impulse alone reaching the muscle.

As to the fact that the subsequent impulses vanish at the beginning of the narcotized part, we have shown plenty of experimental evidence in part I.

In the same stage of narcosis the series of less frequent stimuli (10.0σ interval) produced a tetanus. The reason is quite simple: the first impulse reaches the muscle as in case of the frequent stimuli. The second falls far to the right of AA at 10.0σ after the first. Therefore the second reaches the muscle too. As it does set up an impulse in the narcotized part, the narcotized part recovers from it just as it did from the first

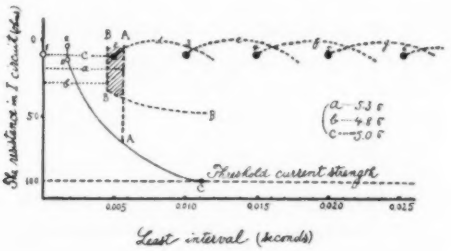

Fig. 15

impulse. This is a matter of course viewed from the standpoint of the all-or-none principle. As to this fact we have reported elsewhere (24). Therefore the power of the second to inhibit the succeeding impulse is the same as the first. It is indicated by a in the figure. It lasts only 5.3σ at this stage of narcosis. The third impulse comes at 10.0σ after the second, and so it reaches the muscle, and so on. Thus a series of stimuli at a frequency of 100 per second (the interval is 10.0σ) produces a tetanus, whereas a series at a frequency of 200 per second produces complete inhibition at the same stage of narcosis. This is nothing other than the "frequency factor."

Some words will be added as notice: if a tuning fork or a usual interrupter is used to obtain tetanic stimuli, then the impulses aroused by them are not, contrary to the expectation, regular in interval, as was observed by examination of the action currents (p. 698). Consequently it may happen that a certain impulse, say the third or the fourth, falls in the relative refractory period due to its preceding impulse and vanishes. Then

it leaves behind a long after-effect and the subsequent impulses may be abolished in turn as in case of complete inhibition. In such a case the muscle contraction, although it is called the "initial twitch" (Anfangszuckung), is somewhat higher than that produced by a single stimulus. We may call such a case "incomplete inhibition." That there are two kinds of so-called initial twitch was mentioned in part I.

8. On the "after-effect." Now there remains the last question to be answered, "can it be possible that the nerve impulse which was extinguished in the relative refractory period because it was below the threshold at that moment would leave behind so long an after-effect?" We have plenty of evidence in favour of it. The results of the experiments made in this laboratory will be briefly cited.

1. If a subminimal stimulus (electrical or mechanical) is applied at a point, X, of nerve, it sets up of course no nerve impulse, but this stimulated

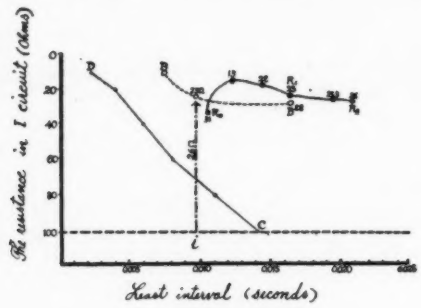

Fig. 16

point X suffers a marked change in its excitability. The threshold is decreased for a very short time (1 to 2σ) immediately after the subminimal stimulus and then is increased. This increase, i.e. inhibitory after-effect, lasts fairly long (about 10σ) until it again becomes normal. This effect is localized at the stimulated point X.

2. A similar effect is obtained with a recovering nerve. If a nerve recovering from the first stimulus is stimulated during its relative refractory period with a second stimulus which is below the threshold at the moment, the recovery curve suffers a similar change, although the second stimulus sets up no nerve impulse. This change of the recovery curve is more marked in narcosis.

3. In the advanced stage of narcosis in which we usually see complete inhibition this inhibitory after-effect is very pronounced. Since it has an immediate bearing on the problem in question we think it worth while to describe one example in some detail. Figure 16 shows the result obtained

in experiment 8. The experiment was conducted as follows: a nerve muscle preparation was mounted in a narcotizing chamber in the usual way. Before narcosis the recovery curve of B (inside electrode) was mapped, CD, with two keys of a Lucas pendulum. The strength of the second stimulus was varied by changing the resistance in the primary cricuit (threshold 100 ohms). Then the narcosis (0.5 per cent cocaine-Ringer) was started. After 165 minutes of narcosis complete inhibition was produced by tetanic stimulation applied at A (outside electrode). At this advanced stage the recovery curve of B was mapped again, BB. It approached a horizontal line, the threshold increasing from 100 ohms to 28 ohms, as in the experiment previously mentioned (fig. 9). It indicates that the narcotized part, B, recovers, at this stage, along the curve BB and it was confirmed that at 9.8σ after the first stimulus the threshold of B was 25 ohms. In other words, the second stimulus having 25 ohms in its primary circuit was just

TABLE 18

NUMBER	ABSOLUTE REFRACTORY PERIOD OF NARCOTIZED PART	INHIBITORY TIME R_1	AFTER-EFFECT R_2
	σ	σ	σ
1	5.5	7.1	11.1
2	5.6	4.8	7.9
3	4.3	7.1	11.1
4	4.4	4.7	7.1
5	4.9	3.9	5.5
6	5.9	7.1	17.0
7	4.5	3.9	4.7
8	7.4	5.5	10.5
9	6.5	4.7	8.0
10	5.3	4.7	8.7

able, being applied at B, to produce summated contraction if applied at 9.8σ after the first stimulus. Therefore at this stage of the relative refractory period the second stimulus having 26 ohms in the primary circuit is below the threshold at the moment, and so it sets up no nervous impulse. The main point in this experiment is to examine the after-effect left by such an apparently ineffective second stimulus. For this purpose three keys of Lucas pendulum were employed, and the change of the recovery curve was determined, interpolating the second stimulus, i, at the above mentioned time and strength. The curve $R_0R_1R_2$ shows the result obtained. It will be seen that the threshold is decreased for a very short time immediately after i and then is increased. This increase, i.e. inhibitory after-effect, is pronounced. For example, the stimulus having 25 ohms in its primary circuit, i.e. the threshold stimulus at the time of the interpolation, was effective only after 15.3σ (R_1 in fig. 16) after the first stimulus, whereas it

was effective as early as at 9.8σ after the first, if applied without the interpolated stimulus i. Therefore the threshold returned to its own value at 5.5σ ($15.3 - 9.8 = 5.5$) after the interpolated stimulus. The time so calculated is shown as "inhibitory time" in table 18. On the other hand the stimulus having 26 ohms (same strength as i) became effective only after 20.8σ, R_2, after the first, whereas it was effective, if without i, at 10.3σ after the first: in other words the stimulus i which, being applied at B, actually fell in the relative refractory period due to the first stimulus inhibited the third of the same strength as long as 10.5σ ($20.8 - 10.3 = 10.5$). The time so calculated is shown as "after-effect" in table 18. Eighteen experiments were made in this way, varying 1, the stage of interpolation in the relative refractory period, and 2, the strength of the interpolated stimulus. However we will be content

TABLE 19

AGENTS (PER CENT USED)		CLASS	AGENTS (PER CENT USED)		CLASS
Narcotic in Ringer solution	Cocaine (0.5–1.0 percent)	I	Toxic substance in Ringer solution	Tetrodotoxin. (0.02–0.03 per cent)	I
	Alcohol (6.0–7.0 per cent)	I		Potassium chloride (0.95 per cent)	II
	Paraldehyde (1.5 per cent)	I		Potassium cyanid (0.4 per cent)	II
	Urethane (2.5–3.0 per cent)	I		Sublimate (0.5 per cent)	II
	Sulfonal (0.2 per cent)	II		Formalin (0.5–2.5 per cent)	II
	Chloral hydrat (4.0–5.0 per cent)	II		Acid. hydrochlor. (N/10)	II
	Bromal hydrat (4.0 per cent)	II	Gas	Chloroform	I
	Ammonia (0.01–0.04 per cent)	II		Alcohol	I
Osmotic pressure	Aqua dest. (hypotonic)	II		Ether	I
	Glucose solution (isotonic)	II		Ammonia	I
	10X Ringer solution (hypertonic)	II		Hydrogen	II
				Acid carbonic	II
Temperature	Heating (40°C)	II			
	Cooling (−4°C)	II	Electricity	Galvanisation	II

with showing only "inhibitory time", "after-effect", and the absolute refractory period of the narcotized part (i.e., the least interval at B) at the moment of determination (table 18). It will be noted too that the inhibitory after-effect left by the interpolated stimulus lasted longer as the recovery curve of the narcotized part (BB in fig. 16) approached, at the time of determination, nearer to a horizontal line. This result is a strong support of the explanation of the Wedensky effect offered by us. It was shown by this experiment that the stimulus which falls in the relative refractory period without setting up any nerve impulse does actually inhibit the succeeding impulse (third stimulus) for so long a time. With this result the decrement or "transitional decrement" has nothing to do. It is an effect strictly localized at the stimulated point. In view of this experimental result, it will be apparent that in the Wedensky effect the subnormal impulse which was

extinguished in the relative refractory period due to the first impulse left behind an after-effect which inhibited the third and so on.

9. Some observations on Wedensky inhibition and their explanation. Besides narcotics, there are many other agents which can produce the Wedensky effect, and, as the following experimental results show, the ease with which it can be produced is different according to the agents. Even among narcotics we see differences. Being examined with a series of stimuli of the same frequency (100 per sec.) some agents produce complete inhibition in an early stage when the contraction height evoked by a single maximal stimulus applied at the outside electrode remains still nearly normal, whereas others produce it only so late that the contraction height is reduced below one third of the normal. Taking as criterion the height of muscle contraction obtainable at the stage of affection at which complete inhibition is first produced, we divided the agents into two classes, I and II.

TABLE 20

NARCOTIC (PER CENT USED)	CONTRACTION HEIGHT IN THE STAGE OF CROSSING (NORMAL AS 1)
Cocaine (0.2 per cent)	Above $\frac{1}{3}$
Alcohol (5.0–6.0 per cent)	Above $\frac{1}{3}$
Paraldehyd (0.5–1.0 per cent)	Above $\frac{1}{3}$
Urethane (2.0 per cent)	Above $\frac{1}{3}$
Sulfonal (0.2 per cent)	Below $\frac{1}{3}$
Potassium cyanid (0.4 per cent)	Below $\frac{1}{3}$
Chloral hydrat (1.0–2.0 per cent)	Below $\frac{1}{3}$
Ammonia (0.01 per cent)	Below $\frac{1}{3}$
Glucose (4.0 per cent)	Below $\frac{1}{3}$
Sublimate (0.09–0.3 per cent)	Below $\frac{1}{3}$

I class: those which produce complete inhibition before the contraction height is reduced to $\frac{1}{3}$ of the normal.

II class: those which produce it below $\frac{1}{3}$ of the normal.

In table 19 are shown the agents tested and their classification.

The main interest lies in considering why it can be so. The thought will arise at once: it is probably due to the difference in the stage at which the curves A and B cross (see fig. 10). The relation between the crossing stage, K, and the contraction height at the moment of the crossing was thoroughly examined in some of the representative agents shown in the above table. The results are summarised in table 20. It will be seen that the results are in good agreement with the expectation. For example, in case of cocaine the crossing, K, occurred always when the contraction height was above $\frac{1}{3}$ of the normal, whereas in case of chloral hydrate the crossing was observed only when the contraction height was reduced below $\frac{1}{3}$ of the normal. Therefore the former might well belong to the I class and the

latter the II. Thus the occurrence of the crossing and the production of complete inhibition go in parallel. Without the former the latter can not be produced.

10. Depth of narcosis and Wedensky effect. It is well known that the more the stage of narcosis is advanced, the more easily Wedensky effect can be produced. However, as far as we know, no one has ever given a satisfactory explanation for this experimental fact. From our point of view, it is simple to explain. From the observations hitherto described, it is evident that for the production of Wedensky effect it is necessary that the second impulse fall in the shaded area between *AA* and *BB* (see fig. 15), or in a wider sense including incomplete inhibition, we may say that it is necessary that a certain impulse fall in the relative refractory period due to the preceding one and vanish. We have shown that as narcosis proceeds the least intervals at *A* and at *B* gradually increase, and after their crossing the difference between them becomes gradually greater (table 8 and fig. 10). It means that the shaded area in figure 15 moves to the right and increases in width as narcosis deepens. In the same manner the relative refractory period due to any impulse increases the duration within which it can abolish the subsequent impulse. Consequently in the advanced stage of narcosis, 1, a second impulse of less frequent stimuli would fall in the shaded area to produce complete inhibition; 2, there are more chances that some impulse would fall in the relative refractory period of the preceding one and vanish. This is the reason why we can produce Wedensky effect with greater ease in an advanced stage of narcosis, choosing the stimulus frequency at random.

"*Intensity factor.*" At a certain stage of narcosis a series of strong stimuli produces "complete inhibition," whereas a series of weak stimuli produces a continued tetanus, although the stimulus frequency is kept unchanged. As to this intensity factor we came to the conclusion that it is due to the difference in the number of nerve impulses set up by the strong and the weak stimuli in question. Therefore the intensity factor is to be attributed, as Lucas (25) and Adrian (26) did, to the frequency factor. By examining the number of action currents we confirmed their opinion on this point. The experiments made in this laboratory will be briefly described.

A nerve muscle preparation was set up in a narcotizing chamber and the tetanic stimuli could be applied at *A*, the outside electrodes. The nerve length between *A* and the upper chamber wall was not less than 30 mm. To obtain the tetanic stimuli an interrupter whose frequency could be varied between 10 and 200 per second was inserted in the primary circuit. The strength of the stimuli was varied by changing the coil distance. At a certain stage of narcosis the coil distance, *i*, was determined at which

complete inhibition was produced and then the intensity was reduced to the coil distance, t, at which a continued tetanus was produced, keeping the stimulus frequency unchanged. i and t were marked on the slide of the inductorium. Then the nerve was cut just outside the upper chamber wall and was led to a string galvanometer so that the action currents could be examined by applying the tetanic stimuli at A. Photographic records of the action currents were taken applying the tetanic stimuli at coil distance i and then at t. The former represents the number of nerve impulses entering the narcotized part to produce complete inhibition and the latter

TABLE 21

NUMBER	NARCOTICS	COIL DISTANCE	NUMBER OF ACTION CURRENTS PER SECOND
		mm.	
1	0.5 per cent cocaine Ringer solution	Inhibition (i) 150	135
		Tetanus (t) 250	87
2		Inhibition (i) 150	158
		Tetanus (t) 250	88
3		Inhibition (i) 150	151
		Tetanus (t) 200	70
4		Inhibition (i) 150	142
		Tetanus (t) 240	74
5		Inhibition (i) 150	142
		Tetanus (t) 240	119
6		Inhibition (i) 150	142
		Tetanus (t) 210	100
7		Inhibition (i) 150	141
		Tetanus (t) 200	119

that producing the continued tetanus. The results are summarised in table 21. It will be seen that there is a marked difference in the number of the action currents (nerve impulses) between two series of stimuli producing respectively complete inhibition and a tetanus, although the stimulus frequency was equal in both cases. Therefore it is highly probable that the intensity factor is due to this difference, but not fully conclusive. Consequently we proceeded to make control experiments as follows. As an example experiment 3 will be described. A nerve muscle preparation was set up in a narcotizing chamber as in the previous experiments. Before narcosis the number of the diphasic action currents was examined

(without cutting the nerve) between *A* and the upper chamber wall, applying the tetanic stimuli at a coil distance of 150 mm. and then at 240 mm. They were respectively 142 and 74 per second. Then the narcotic (0.5 per cent cocaine-Ringer) was applied and we waited till the stage was reached at which the tetanic stimuli of the coil distance 150 mm. produced complete inhibition. At this stage the tetanic stimuli of the coil distance 240 mm. produced a tetanus. As soon as this was confirmed, the stimulus frequency was reduced, adjusting the interrupter to 70 or 75 per second, and at this new frequency the tetanic stimuli were applied at 150 mm. coil distance. They produced a marked tetanus. Thus these new stimuli were of the same intensity as those which produced complete inhibition, and of the same frequency as the nerve impulses producing tetanus. However, whether these stimuli actually produced nerve impulses in the number required, needs further confirmation. Therefore, after this procedure was finished the action currents were again tested. It was found that these

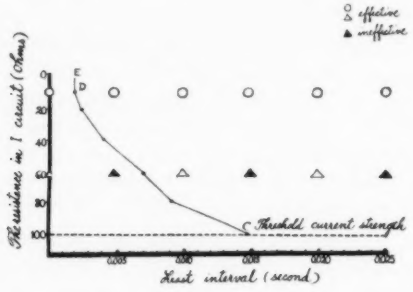

Fig. 17

new stimuli set up 71 impulses per second. Thus it was confirmed that the intensity factor is a frequency factor in different form.

As to the reason why the difference in the strength of the stimuli produces a difference in the number of nerve impulses, a glance at the recovery curve of the stimulated point will suffice to make it clear. In figure 17 the circles represent a series of strong stimuli at 200 per second and the triangles that of weak stimuli of the same frequency. Of the former the second is effective because it falls above (to the right of) the recovery curve *CDE* and the nerve recovers from it just as it did from the first. Therefore the third is effective too and so on. On the contrary, the weak second stimulus is ineffective because it falls below (to the left of) the recovery curve; in other words, it is below the threshold at the moment. The third, falling above the curve, is effective and the nerve recovers from it just as it did from the first. Consequently the fourth is again ineffective. Thus weak stimuli are ineffective in producing a series of impulses of high frequency.

SUMMARY

1. The recovery curve of the narcotized part was studied, and it was shown that the curve approached, as narcosis deepened, almost to a horizontal line, indicating prolonged absolute refractory period and heightened threshold in the relative refractory period.

2. To know the depth of narcosis necessary to produce complete inhibition the least intervals were determined at *A* (normal part outside) as well as at *B* (narcotized part) through the whole course of narcosis. It was shown that in the early stage of narcosis the least interval at *A* increased more slowly than that at *B*, and from certain stages the former increased more rapidly and overtook the latter. Therefore the curves *A* and *B* cross (see fig. 10). And it was shown that complete inhibition could never be produced until this stage of narcosis at which the curves cross, however frequent the tetanic stimuli might be.

3. The reason why the curves *A* and *B* cross is discussed. They cross because the second impulse started from *A* vanishes in the narcotized area by falling into the relative refractory period left by the first impulse.

4. For the production of complete inhibition such a depth of narcosis is necessary that the second impulse would be extinguished on account of the heightened threshold in the relative refractory period due to the first impulse. This second impulse, although itself extinguished, leaves behind an "after-effect" which lasts long enough to inhibit the third and so on.

5. The duration of the inhibitory "after-effect" left by the second (extinguished) impulse was measured. Similar experiments were made to measure the inhibitory "after-effect" left by the third (extinguished) impulse.

Furthermore experiments were described in which the narcotized part was directly stimulated during the relative refractory period, with a stimulus which was below the threshold at the moment. The inhibitory "after-effect" left by such an apparently ineffective stimulus falling actually in the relative refractory period was measured. It was sufficiently long, in an advanced stage of narcosis, to account for the "after-effect" left by the extinguished impulse.

6. The conditions necessary to produce complete inhibition are 1, the curves *A* and *B* cross; 2, the second impulse falls in the shaded area between *AA* and *BB* (fig. 15).

7. The reason why there is a marked difference in producing Wedensky inhibition according to the agents used, and also the reason why the more advanced is the stage of narcosis the more easily can Wedensky inhibition be produced, were explained.

8. The "frequency factor" as well as the "intensity factor," which constitute the so-called "paradoxes Stadium" in the sense of Wedensky were explained.

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The explanation of the third stage described by Wedensky (i.e., Hemmungsstadium) was completed, too. It will be published before long.

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